Tools for diagnostics, molecular definition and therapy development for chronic inflammatory joint diseases

Description

The invention refers to tools for diagnostics, molecular definition and therapy development for chronic inflammatory joint diseases and other inflammatory, infectious or tumourous diseases. These tools are based on genomic data (Genomics), proteomic data (Proteomics) and immunological data (Immunomics) in the analysis and therapy development for chronic joint diseases. The invention is based both on the use of gene sequences and deduced mRNAs and proteins and on the use of antibodies being specific for the deduced proteins for characterizing inflammatory rheumatoid and non-inflammatory rheumatoid joint diseases, autoimmune diseases and infectious diseases. Starting from the investigations one can derive etiologically important pathogenicity principles of the hitherto unexplained chronic inflammatory joint diseases. Moreover, one can construct interpretation algorithms for the classification, prognostic evaluation and therapy optimization of these joint diseases, and moreover one can draw conclusions for novel therapeutic strategies and therapeutic targets.

Overview of the prior art

Technical Problem

The etiology of chronic inflammatory joint diseases is not yet understood. The rheumatoid arthritis (RA – see list of abbreviations following the examples) is the classic example for these diseases. Major processes of the disease take place in the synovial membrane, which is altered in an inflammatory manner, thereby leading to a chronic joint lesion. The clinical picture observed is very heterogeneous, suggesting, that one is faced with several entities showing the common symptom of destructive synovitis. These diseases also have to be understood as systemic diseases, in which a multitude of changes is observed in the blood and which sometimes result in severe organic manifestations.

Overactive inflammatory activities due to dysregulations in the inflammatory cascade are discussed as major pathogenic mechanisms. Furthermore, autoimmune reactions have been described, which suggest a role of the specific humoral and cell-mediated immune system in the pathogenic process. However, also other mechanisms like enzymatic tissue destruction,

cell and tissue proliferation or regeneration are discussed, these factors also potentially playing a crucial role in pathogenesis.

It was so far not possible to finally determine, if these mechanisms of pathogenesis are the sole and exclusively relevant ones. It is furthermore unknown, which parameters are able to simultaneously encompass all these changes. In consequence of the insufficient pathophysiological understanding, numerous therapeutics are available, the major examples of which however only follow one main therapy concept:

Focusing on the common symptom of excessive inflammation, the current therapy thus aims to suppress inflammation. So-called basal therapies display an immunomodulating and disease-modifying character. They interfere with basal mechanisms of cellular metabolism and cellular activity (e.g. Methotrexate, Azathioprine). The comprehensive principles of the molecular mechanism of these therapies in the joint diseases however are incompletely understood. In consequence, there is a lack of respective parameters for controlling the therapeutic efficiency of single basal therapies in a differential and specific manner in the individual case.

Previous Tools

Patients with joint diseases are nowadays evaluated according to the following criteria in the clinical routine: reported progression of the disease (anamnesis), clinical picture (disease pattern observed in the joints, organic manifestation), parameters of inflammation (unspecific inflammatory parameters observed in serum electrophoresis, sedimentation rate, and C-reactive Protein), autoimmunogenic parameters (rheumatoid factor, antinuclear antibodies and a few specific auto-antibodies like anti-Ro, -La, -U1RNP, -Sm, -Histone, -Scl70, -Centromere, -dsDNA, -phospholipid-antibody), genetic predisposition based on HLA-markers (DR4, B27, DR3), image forming (destructive alterations in the X-ray picture of the joints), extended organ diagnostics by means of routine parameters of laboratory diagnostics (liver enzymes, muscle enzymes, kidney retention values) and, if favorable, further techniques of sonography, radiology and magnetic resonance tomography. These only allow for very limited predications concerning the aggressiveness of the disease to be prognosticated or concerning the concrete expectations of success of a basal therapeutic in the individual patient. Moreover, the diagnostic criteria are nowadays not designed for sufficiently

classifying the diversity of manifestations in the most common arthritic disease, the RA (1, see references following the examples). Especially in the early phase of the disease, diagnosis is difficult and uncertain. After an endurance of the disease of just one year however, the majority of the patients already suffers from irreversible joint lesions. It is known from early-stage arthritis studies, that a diagnosis being earlier confirmed and followed by an adequate therapy goes along with essential improvements concerning the long-term development of the disease. Novel methods and criteria, integrating molecular features beyond the clinical picture are thus extremely necessary.

Also the progress monitoring of the therapeutic success is hitherto accomplished by means of the above mentioned methods of diagnosis. Many of these parameters only change very slowly. They require many weeks to months of observation in order to come to a conclusion, if the chosen therapeutic is effective. Often the therapeutic has to be changed due to insufficient amelioration and progression of the disease. Healing of the diseases is generally impossible by using the therapeutics currently available.

Experimental Approaches

There exist many experimental approaches beyond the established tools in order to improve the diagnostics especially of RA.

They refer to the search for key proteins, which 1.) maintain or prevent the progression of inflammation in a central position, 2.) are decisively taking part in the enzymatic destruction of the cartilage and bone matrix or which inhibit the responsible enzymes, or 3.) can induce regenerative and reparative processes or inhibit their antagonists. Here for example, the role of the inflammation-mediating cytokines Tumour Necrosis Factor (TNF-) alpha and Interleukin (IL-) 1 beta has proven to be essential and has thus introduced respective therapeutic approaches into clinical use. Although an inhibition of TNF-alpha can in many cases ameliorate a RA being not sufficiently affected by common tools, these positive results however do not lead to a healing of the disease. Partly, the inhibition is such strong, that infections or even septic complications arise and a sufficient control of arthritis is nevertheless not accomplished. This suggests, that the TNF-alpha-mediated pathway of inflammation is at least not the only central pathogenic mechanism of the disease. Besides the two mentioned cytokines, the role of numerous other signal substances in the pathogenesis of arthritis is

under investigation. In addition, therapeutic intervention increasingly focuses on the corresponding intracellular signal pathways.

Moreover, the matrix metalloproteinases and cathepsins are in the center of the enzymatic destruction of bone and cartilage.
Investigations of regenerative mechanisms are just at the beginning of research. In the first place one has to mention signal substances belonging to the Transforming Growth Factor (TGF-) beta-family. A large number of them plays a crucial role in the development of the locomotor system. First investigations on synovial tissue and cartilage have shown, that members of this group of growth factors and morphogens are also produced in the adult synovial tissue. For inflammatory joint diseases, we were able to show in our own investigations, that some of these factors obviously show a relative decrease. Furthermore, it was able to be shown for Bone Morphogenetic Protein (BMP-) 7, that the cellular invasion into developing artificial cartilage tissue was suppressed (2).

Many of the mentioned factors and enzymes are also to be found in other joint diseases like osteoarthrosis or the reactive arthritic diseases and therefore - being regarded for themselves - do not constitute a specific diagnostic parameter.

The experimental approaches also focus on the fact, that auto-reactive T- and B cells arise in RA, which is accordingly classified into the group of autoimmune diseases. This classification goes back to the discovery of the so-called rheumatoid factor, an auto-antibody, which is directed against immunoglobulin G. Rheumatoid factors however only occur in about two thirds of the RA-patients, but are also present in other rheumatoid and non-rheumatoid diseases and even in up to 5% of the healthy population (even to a higher degree with increasing age). The occurrence of rheumatoid factors seemingly is a physiological reaction of the body under certain pathological conditions, like e.g. the bacterial endocarditis. Auto-reactive B cells with a specificity for IgG are seemingly present in a major part of the population and can be activated by different mechanisms. The term "rheumatoid factor" was nevertheless maintained, since it only offers a diagnostic and prognostic meaning for RA.

The same characteristics however do also qualitatively apply for nearly all auto-antibodies, which are hitherto known for RA: the frequency of positive patients is significantly less than 100% and the disease specificity in part is also significantly less than 100%. The pronounced

clinical heterogeneity of RA in respect to the disease pattern, the intensity of inflammation and the intermittent character is thus in parallel to a heterogeneity of the immunologically dysregulated processes. This clinical and immunological heterogeneity also supports the speculation, that the "rheumatoid arthritis" may be a general term for different disease entities. A typical example for this is the differentiation between the RF-positive and RF-negative (RF - rheumatoid factors) RA, whereat the first is said to have a more severe progression with a higher destructive potential and a systemic humoral activity. The term "seronegative" erroneously implies even the absence of any auto-antibody. However, neither the rheumatoid factor nor anyone of the other known autoreactivities could be confirmed as an etiological cause for the rise of RA or one of its postulated subforms or progress forms.

Auto-antibodies are used for diagnostic classification in case of other rheumatoid autoimmune diseases like the collagenoses with systemic Lupus erythematodes (SLE) as their major member. A primary pathogenicity of these auto-antibodies is constantly and repeatedly discussed. It is certain, that a high titer of auto-antibodies in combination with an unscheduled, excessive release of auto-antigens during an intermittent episode of the disease and the subsequent formation of immune complexes and complement activation is associated with organic lesions, especially of the kidney, and with vasculitic features. The role of the auto-reactive B- and T cells in RA however is not determined. Instead, novel auto-antigens are evermore described as targets of an autoreactive immune response in RA. Some of these antigens are well characterized in respect of their biochemistry and antigenic features, others however are only understood in respect of a few parameters. Some of these auto-antibodies were very promising for their discoverers, since the B and/or T cell-response appeared to be highly specific for RA. The interest in these antibodies however always quickly vanished, when the same autoreactivities were also detected in other autoimmune diseases. Meanwhile, numerous T cell-associated autoreactivities have been discovered for RA, only a very few of which however are specific for RA.

Heat shock Proteins

The RA has soon been suspected to constitute an infectious disease. Therefore, a diversity of xenogenous antigen sources - in most cases of microbial or viral origin - was investigated in order to detect potential pathogens acting as triggers of autoreactivity. One of the potential RA-inducing agents was *Mycobacterium tuberculosis*, since in the animal model it induces

the adjuvant-arthritis, a disease being similar to human RA in certain aspects. This experimental disease was also able to be induced by the mycobacterial heat shock protein 65 (mt-Hsp65) or by T cells, which are specific for this antigen. Heat shock proteins support native proteins in developing their correct three-dimensional structure, thereby creating tertiary and quaternary structures. mt-Hsp65 is homologous to the essential Hsp60 in mammalian species. Reports about mt-Hsp65-specific T cells and antibodies in the synovial fluid of RA-patients suggested, that the strongly homologous human Hsp60 would be recognized as an antigen in RA-patients. These antibodies however, are not specific for RA: They also occur in patients with Reiter's syndrome, SLE and active tuberculosis, but also in healthy persons.

Although the reactivity against mt-Hsp65 does not seem to play a dominant role in RA, human Hsp60 might nevertheless be important in the pathogenesis of RA: In its amino acid sequence, human Hsp60 - in regions of 11 to 22 amino acids - has an identity with proteins like cytokeratin and Hsp90. It is thus conceivable, that autoreactive T cells or antibodies against these proteins originally result from a natively occurring - but strictly regulated - Hsp60-reactivity.

Dna J

Dna J, the bacterial stress protein having homology to mammalian Hsp70, provides the amino acid sequence QKRAA, better known under the designation "*Shared Epitope*", which confers predisposition to RA (3). This epitope also occurs in the protein gp110, which is encoded by the Epstein-Barr virus (EBV). Dna J is the target of autoreactive T cells under the conditions of RA, but not in the healthy patient (4). Although it is still unknown, in which way Shared Epitope confers RA-predisposition, one conceivable mechanism may be the generation of the Shared Epitope-peptide from non-MHC-proteins and the subsequent presentation on MHC class II-molecules, thereby inducing an immune response against foreign (EBV-gp110) and self (MHC class II).

EBV-encoded nuclear antigen

Epstein-Barr virus (EBV) has soon been suspected to cause RA, although it has just recently been possible to detect this virus in the synovial fluid of RA-patients. An antibody, directed

against the EBV-encoded nuclear antigen (EBNA-1), showed strong reactivity with a p62-protein from synovial mesothelial cells in patients with RA. EBNA-1 contains a glycine-alanine-rich repeat sequence (IR-3), which is recognized by auto-antibodies in patients with RA, SLE, systemic sclerosis (SSc) and infective mononucleosis, but also in healthy individuals in comparable frequency. EBNA-1 shows cross-reactivity with numerous human proteins, typically via the IR-3 sequence. Among these, essential examples are p62 and p542, whereat the latter is mainly recognized by antibodies from patients with infective mononucleosis, but also from RA-patients. P542, due to its high sequence identity with the mouse hnRNP designated "Raly" and similarities with the human hnRNP C2, has recently been identified as the 71k component of hnRNPs.

Sa-antigen; Filaggrin, citrullinated peptides/proteins

The Sa-antigen (5) and filaggrin are two recently discovered antigens, which are not present in the inflamed joint, but attracted attention because of the highly RA-specific immune response. The Sa-antigen is a 50k-protein derived from human spleen and placenta. Sa-specific antibodies occur in 43% of the RA-patients and have a disease specificity of 78% to 99%. Filaggrin is a 42k-protein, which is responsible for cross-linking intermediary filaments, in particular cytokeratin, and which is present in the endothelium. Filaggrin-specific antibodies are apparently the same as the "antiperinuclear factor", which was described a long time before, and as the so-called anti-keratin antibodies. The major determinant of the epitope(s) being recognized by the anti-filaggrin antibodies is citrullin, a post-translationally modified arginine (6, 7). The sensitivity of these antibodies is between 36% and 91%, and the specificity is between 66% and 100%. Although filaggrin only occurs extra-articularly, citrullin meanwhile has been successfully detected also in synovial cells.

Collagen II

Collagen type II is a major component of the joint cartilage and thus seems to be predisposed as an auto-antigen for RA. Accordingly many studies have dealt with the role of the collagen-specific immune response. Mouse T cells reacting with the bovine collagen type II are specific for an epitope, which also occurs in human collagen II and which furthermore overlaps with an important T cell epitope from mice suffering from collagen-induced arthritis. Collage type II is a component of the extracellular matrix, which produces triple helices from

identical tropocollagen subunits, which themselves are processed from the even larger procollagens. B cells having specificity for collagen seem to occur in the inflamed joints of RA-patients in a more pronounced manner. T cells being specific for Collagen II occur as well in RA-patients as in healthy individuals.

The collagen reactivity attracted particular attention within the scope of the studies of oral tolerance in RA. In the animal model, oral tolerance can be induced by means of antigens occurring in the compartment of the (autoimmune) inflammation, but not being necessarily involved in the inflammatory process themselves. If such an antigen is orally applied, T cells having specificity for the fed antigen are apparently tolerated and are then capable to produce the so-called Bystander-Suppression via suppressive factors, like e.g. IL-10 and TGF-β, in another place, namely the inflamed joint. T cells being such specific for collagen II were intended to downmodulate the inflammation in RA. However, three placebo-verified double blind studies of oral tolerance did not reveal a significant improvement of disease activity, when collagen II was applied. A similar result also applies for clinical studies with peptides from Hsp65 (Subreum).

Chondrocyte antigen 65 (CH65)

Chondrocyte membranes were reported to be a target of autoreactive T cells in RA- and arthrosis-patients (8), whereas T cells of normal donors did not show such a reaction. Moreover, chondrocyte membranes are recognized by auto-antibodies in 70% of the RA-patients. The respective antigen is the cartilage-specific CH65, which shows a sequence similarity to mycobacterial Hsp65 and certain cytokeratins. CH65 displays a high proportion of glycine, similar like, but not identical with Hsps. Although the sequences are similar to those of keratins, they are nevertheless completely untypical for them. Such similarities allure to arrive at the idea of a molecular mimicry between human/mycobacterial Hsps and other proteins. However, no cross-reactivity has been found between the monoclonal antibodies, which are specific for CH65, cytokeratin or Hsp65. T cell reactivity was just investigated against unpurified chondrocyte membranes.

HC gp39

In the synovial fluid, numerous antigens occur, which were only tested in little groups of patients and controls. One example is the Human Cartilage-Glycoprotein (HC gp39), an important product, which is secreted by articular chondrocytes, synovial cells, macrophages of late stages of differentiation, and by neutrophils. The gp39-level in patients with a degenerative joint disease is increased in the serum and the synovial fluid in comparison to healthy individuals. Later it was shown, that an increased titer not only occurs in case of osteoarthrosis, but also in case of colorectal carcinoma, alcohol-induced liver cirrhosis and breast cancer. gp39 not only has a role in reorganising tissues and degrading the extracellular matrix, but it also is a target of autoreactive T cells in RA. Accordingly, also peptides from the gp39-sequence were tested to bind HLA-DR4 (DRB1*0401) and to stimulate T cells. gp39-reactive T cells were detected in 8 of 18 RA-patients and 3 of 11 healthy individuals. In the animal model, an immunization of Balb/c-mice leads to a chronic arthritis with intermittent episodes, which again was able to be healed by a nasal application of gp39.

Rheumatoid factor

The best known auto-antigen in RA is at the same time not tissue-specific, but can occur nearly ubiquitously. It is the immune globulin G (IgG) as the target of further antibodies, the so-called rheumatoid factors (RF). The rheumatoid factor is still the only serological parameter, which is comprised within the criteria of the American College of Rheumatology (ACR-criteria). The pathological relevance of RF for RA is still controversially discussed, since RF also occurs in patients with SLE, Sjögren's syndrome, endocarditis, liver diseases and even in healthy persons. The RF-titer is not strictly correlated with the clinical or serological activity of RA or with the degree of joint destruction.

hnRNP A2-Protein (RA33)

The A2-protein belonging to the human nuclear ribonucleoproteins (hnRNPs) is a ubiquitous protein, which was originally described as RA33 auto-antigen. In the following, both its identity with the A2-component and its reactivity with sera from patients suffering from SLE, mixed collagenoses (Mixed Connective Tissue Disease; MCTD) and other diseases, were shown. A2 is present as a complex with numerous other factors, which together represent the hnRNPs in the nucleus. The exact function of A2 is unknown, although a function in splicing the human nuclear ribonucleic acid (hnRNA) is supposed. Accordingly, A2 provides two

RNA-binding domains and a nuclear import/export signal. Antibodies in RA and SLE are directed against the region between the RNA-binding domains, whereas those in MCTD-patients (Mixed Connective Tissue Disease) recognize a discontinuous epitope, which is comprised of both RNA-binding domains. It is not yet clear, how the immune system gets into contact with A2. From the view-point of the homunculus however, the hnRNPs are good candidate-antigens for RA. Up to now however, one can only speculate, that A2 - under certain circumstances - arrives at the cellular surface, e.g. during the cell decay in the course of an inflammation.

Calpastatin

Calpastatin is a ubiquitous cytoplasmatic protein having a molecular mass of 72 k and four inhibitory domains for calpains. Calpains comprise a family of cysteine-proteases, which are suspected to be involved in the joint destruction in rheumatoid diseases. Calpains occur in the cytoplasm and are stringently regulated by calcium ions for activation and by calpastatin for inhibition. After cell activation, calpastatin occurs also extracellularly und is thus accessible for antibodies. Calpastatin is recognized by auto-antibodies in patient with RA, SLE, polymyositis/dermatomyositis (PM/DM), MCTD, activated arthrosis and venous thrombosis. In the animal model of calpastatin-deficient rats, no symptoms of arthritis are able to be induced. Calpastatin, calpains and calpastatin-specific antibodies are present in the inflamed joints of RA- and OA-patients and might thus be involved in the pathogenesis of these diseases.

Calreticulin

Calreticulin is an ubiquitous protein of the endoplasmatic reticulum (ER), which - under certain circumstances - also occurs in the nucleus, the cytoplasm and on the cellular surface. It constitutes a highly conserved Ca^{++}-binding protein. Calreticulin is the target of auto-antibodies in a number of different diseases of auto-immunological or inflammatory origin, mainly in SLE and onchocercosis, but also in RA. Furthermore, the RA-associated haplotype DR4Dw4/DR53 binds a peptide from Calreticulin.

BiP (Heavy Chain Binding Protein)

A further promising target antigen for the homunculus of RA is the ubiquitous BiP (Binding Protein), which was originally described as Heavy Chain Binding Protein, since it interacts with the heavy chains of immunoglobulins. BiP itself is a resident ER-protein and possesses a peptide sequence preventing the protein from being exported under normal conditions. Meanwhile is has been revealed, that BiP is a so-called molecular chaperon, which in this role interacts with most of the proteins, which are introduced into the endoplasmatic reticulum (ER) and enter the secretory pathway. Beyond this essential functional feature, BiP is overexpressed under the effect of stress factors like heavy metal ions or agents affecting the level of calcium ions in the cell or the integrity of protein biosynthesis. Under these conditions it can even be detected within the nucleus, but also on the cellular surface.

BiP is a target of auto-reactive antibodies and T cells in 66% of RA-patients; it was originally described as p68 in the context of RA. The disease specificity of these auto-antibodies is 99% and thus extremely high. The antigen is O-glycosylated and it is supposed, that this modification might have a regulatory function like mono-O-GlcNAc has in many other proteins. In these proteins, the switch from the O-GlcNAc to the O-phosphate-modification is coupled with a change of the state of activation or of the cellular compartment. In a similar manner, a stress-induced shift of BiP from the ER to the nucleus or to the cellular surface might be of pathogenic relevance. The presence of BiP on the cellular surface, which is rather untypical, might serve as a signal of alarm or activation for other cells, and also for cells of the immune system. In RA such an activation may occur by a local infection or by a tissue being otherwise deteriorated by inflammation. In consequence of the cell- or tissue damage, BiP might arrive at the surface of injured cells, where it then becomes a target of auto-reactive T cells. There exist hints, that these BiP-reactive T cells also occur under natural conditions, under which these T cells are then downregulated by regulatory T cells after the inducing conditions have ceased. The regulatory cells are antigen-specific and HLA-restricted. Thereby, the HLA-restriction of regulatory T cells is apparently distinct from the HLA-restriction of effector T cells and allows to be specifically inhibited. In this context, the epitope O-GlcNAc might again have a crucial role: It is well conceivable, that this epitope is not only a target of the auto-antibody response, but also of the T cell response.

A further protein, which was isolated from the synovial fluid, the function of which however largely goes beyond this compartment, is the p205-antigen. It is a target of autoreactive T-cells in RA-patients. P205 is also expressed in the synovial membrane and probably constitutes the antigen with the highest T cell stimulating capacity in RA at all, partly reaching the proliferation rate, which can be obtained by means of synovial fluid or even by means of the lectin phytohemagglutinin (PHA). The function of the p205-antigen is still unknown. However, it contains a sequence of 11 amino acids, which is identical with a section from IgG, namely within the region between the constant domains C_H2 and C_H3, a region, in which the binding of rheumatoid factors takes place. This region of p205 is both bound by monoclonal rheumatoid factors and also recognized by autoreactive T cells. Furthermore, p205-specific T cells, when being stimulated by cognate antigen, have a supportive effect on B cells in the secretion of rheumatoid factors. It thus has to be assumed, that herewith for the first time an antigen has been discovered, which possesses T cell reactivity and is furthermore capable to support IgG-specific B cells in affinity maturation. In contrast to this, a T cell reactivity against intact IgG or IgG-fragments was not able to be found so far. Possibly, the amino acid sequence of p205 might constitute a peptide, which *in vivo* is not or not sufficiently produced during the processing of IgG. Thus it seems probable, that the auto-reactivity against p205 induces the production of rheumatoid factors in RA.

This summary of RA-associated autoreactivities shows, that many different auto-antigens become targets of the immune system during the process of RA. These auto-antigens to different degrees also become targets of the immune system in case of other rheumatoid and non-rheumatoid diseases and even in the healthy state. Is thus has to be stated, that - according to the present knowledge - no autoreactivity by itself is suitable to improve the diagnostics of RA, neither in the early state, nor in its course or for monitoring a respective therapy.

Character of the invention

The invention has the object to improve and support the diagnosis and therapy of chronic inflammatory joint diseases. This object is achieved by providing the "Tools for the diagnostics, molecular definition and therapy development for chronic inflammatory joint diseases" and other inflammatory, infectious or tumorous diseases. These tools are described in the following.

High-Throughput methods like DNA-array or protein array technology allow for the simultaneous detection of a large number of different parameters (9). Gene expression can be analyzed on the mRNA level by means of DNA-arrays via the hybridization of labeled RNA- or cDNA-samples, and on the protein level by arrays comprising selected protein-specific antibodies (10). Moreover, immunologic reactivities can be accessed by arrays comprising selected antigens (11).

At first, it is necessary to define the genes and proteins, which are relevant for the disease, and which are thus employed for the evaluation.

The tools according to the invention, being designed for diagnostics and therapy development for inflammatory joint diseases, are based on a such defined selection of parameters (table 1 and 2). Employing the genes given herein for a gene expression analysis by an array-method allows for a fundamentally new diagnostic approach.

For DNA-arrays intended for the determination of specific mRNA expression patterns in arthritic diseases, the genes given in table 1 can be employed in their entirety, as well as all of the genes, which are coding for proteins mentioned in table 2. Moreover, one can employ genes or partial sequences of individual genes or a selection of the genes/partial sequences given in table 1, as well as genes or partial sequences of individual genes/partial sequences or a selection of genes/partial sequences, which are coding for the proteins mentioned in table 2.

For characterizing the autoimmune reactivities, the proteins mentioned in table 2 can be used in their entirety, as well as proteins being encoded by the genes given in table 1. Moreover, also a limited selection of these proteins, selected parts of the proteins (in the form of oligo-peptides or polypeptides) or modified forms thereof may be employed. On the protein level, one also and in particular has to consider posttranslational modifications (e.g. glycosylation, phosphorylation, etc.), which can be relevant for a distinction between rheumatic diseases. The proteins, partial protein sequences and modified proteins and modified partial protein sequences are - individually, in groups or altogether - applied on a carrier matrix, which is suitable to test the patient's antibodies for their reactivity against one or several of these components. In consequence, one obtains a profile of reactivities or non-reactivities for a patient. The crucial difference between the prior art diagnostics and the diagnostic approach

presented herein is the determination and analysis of one single auto-reactivity in each case in the prior art and the determination and analysis of a multitude of auto-reactivities according to the invention. The invention makes use of the unexpected finding, that combining several auto-reactivities - which are insusceptible when regarded alone - to one or more profiles, allows for a differentiation, because this approach may e.g. distinguish between a RA and a non-RA (i.e. other rheumatic diseases and non-rheumatic diseases and the healthy state) in 100% of the cases. The classification into distinct profiles is accomplished via a suitable algorithm, in an optimal form via a self-learning algorithm, which is capable to also incorporate later findings.

For the determination of protein expression patterns, array systems have been developed from protein-specific antibodies. By labelling the proteins from a protein extraction of a sample, these proteins can be quantitatively determined after having specifically bound to the corresponding antibody on the array (10). Accordingly, defined as a molecular tool in the sense of the invention is an array, which is comprised of different antibodies or molecules with a comparable protein-specific binding behaviour, being designed for the determination of all proteins or selected proteins being deduced from the genes of table 1 or for the determination of all proteins or selected proteins from table 2.

The diagnostic procedure uses biopsies from the synovial tissue, synovial fluid, blood cells, serum or plasma for the different array analyses. In this procedure, the humoral autoreactivities can be analysed in the liquid samples, the cellular autoreactivities in the blood or synovial tissue cells. The protein expression can be analysed in all of the mentioned samples, the gene expression on the mRNA level in the synovial tissue, in cells of the synovial fluid or in blood cells.

For the analysis by means of DNA-arrays, RNA is extracted from the tissue or from the cell samples derived from blood or the synovial fluid. A sample for the DNA-array hybridisation is prepared under the employment of standard protocols for amplifying (12) and labelling the derived cDNA or cRNA (13).

The genes mentioned in the table, via their known sequences (see accession number GeneBank - http://www.ncbi.nlm.nih.gov/) provide the basis, starting from which specific probes are derived for every gene. These probes are combined in an array, either by applying

the prepared probes by specific printing processes (14) or by site-specific synthesis like in the photolithography on a solid phase (15, 16).

Hybridising of the labeled sample on the array provides quantitative signals via the site- and gene-specific binding, whereat these signals can be translated into an expression profile/-pattern. These patterns are correlated with established methods of evaluation, including the histological features and the classification. By an additional comparison with different joint diseases like osteoarthritis, psoriasis-associated arthritis, reactive arthritic diseases and other, partly also non-differentiated arthritic diseases, this allows for dividing the patients into different groups according to the respective expression profile.

Novelty of the approach

In order to define trustworthy parameters for the array analysis, which allow for a classification and evaluation of the joint diseases, extensive comparative studies were performed. For this aim, different joint diseases were taken into consideration and a novel combination of different methods, partly complementing each other, was chosen.

Thus, synovial tissue from RA, osteoarthrosis and healthy joints was analysed. In order to accomplish a differential analysis of gene expression, at first the "representational difference analysis" (17, 18) was performed. This technique offers the advantage, that all mRNAs being present in the sample are encompassed, even when their sequence is yet unknown. As a drawback, it leads to an intensive selection of the most strongly pronounced differences of expression. Complementary thereto, we also tested the gene expression by means of two different methods of DNA-array-hybridisation, on the one hand on cDNA filter-arrays (19), on the other hand on oligonucleotide micro-arrays (US patents No. 5,445,934; 5,744,305; 5,700,637 and 5,945,334, and furthermore EP 619321 and 373203). These micro-arrays, according to the current state of knowledge, allow to consider nearly all known human genes and to perform a comparative analysis of expression between the tissue samples for each of these individual genes. Finally, the differential gene expression for selected genes was verified in a lager sample collective by means of semiquantitative polymerase chain reaction (PCR, real-time PCR).

Furthermore, tissues were characterized histologically and - according to the histological classification - also compared to the respective differential gene expression pattern. The genes given in table 1 were identified as the differentially expressed genes both between the different chronic joint diseases and in comparison to normal synovial tissue. Thus, these genes are significant for characterizing the chronic joint diseases.

Thus, there also exists a novelty in the selected approach used to identify the relevant genes. The list of the identified genes furthermore shows, that most of the genes have so far not been correlated with inflammatory rheumatoid joint diseases, and it also shows novel evaluation criteria for the diagnostics, investigation of pathophysiology and treatment of chronic joint diseases.

The characteristics of the invention are disclosed and specified by the elements of the claims and by the description, whereat both single characteristics and also several characteristics in the form of combinations constitute favorable embodiments, for which a legal protection is applied for by this specification. These characteristics are comprised of known elements - the genes or partial sequences mentioned in table 1 and the genes and partial sequences coding for the proteins mentioned in table 2 - and novel elements - the novel tools being based on the employment of a defined selection of parameters (tables 1 and 2) -, which in their combination lead to the tools according to the invention, and which, under the employment of the mentioned genes for the gene expression analysis in the array method, allow for a basically new approach of diagnostics and therapy development in inflammatory joint diseases.

The tools according to the invention are based on the employment of a high-throughput method of (micro-) array hybridisation and/or a high throughput method using techniques of the polymerase chain reaction for (semi-)quantification.

They are furthermore characterized in that they are based on the use of a labeled sample derived from a patient and the use of a second, differently labeled control sample, which is used for a comparative double hybridisation to a (micro-) array together with the patient sample (comparative red/green hybridisation). The samples may also be analysed on separate arrays and compared thereafter.

According to the invention, these are tools for diagnostic purposes, which are based on the employment of

- individual, a selection of, or the entirety of the proteins or peptides deduced from the gene sequences mentioned in claims 1 to 3,

- individual proteins, a selection of proteins of all proteins mentioned in table 2, and

- partial sequences derived from individual proteins, from a selection of proteins, or from all proteins mentioned in table 1.

They include proteins or partial protein sequences, which have sequences being identical with those of the deduced proteins of table 1 or with those of the proteins mentioned in table 2, or display a respective sequence identity of at least 80%. They are furthermore characterized in that they are based on the use of

- High-throughput methods in the analytics of protein expression (high definition two-dimensional protein gel electrophoresis, MALDI techniques),

- High-throughput methods in the field of the protein spotting techniques (protein arrays) designed to screen for auto-antibodies as a diagnostic tools for inflammatory joint diseases and other inflammatory, infectious or tumorous diseases in the human,

- High-throughput methods in the field of the protein spotting techniques (protein arrays) designed to screen for autoreactive T cells as a diagnostic tools for inflammatory joint diseases and other inflammatory, infectious or tumorous diseases in the human, and

- Non-high-throughput methods in the field of the protein spotting techniques designed to screen for autoreactive T cells as a diagnostic tools for inflammatory joint diseases and other inflammatory, infectious or tumorous diseases in the human.

The tools according to the invention are furthermore based on the employment of

- antibodies, which are specific for proteins or partial sequences mentioned in claims 6 to 9, and

- the respective homologous sequences of another species for the analytics in animal experiments or for the diagnostics in animals with inflammatory joint diseases and other inflammatory, infectious or tumorous diseases.

The tools according to the invention are useful as diagnostic means for the detection of genetic alterations (mutations)

- in the genes or the regulatory sequences (promoter, enhancer, silencer, specific sequences for binding further regulatory factors) of the genes mentioned in claims 1 to 3, and

- in the genes or the regulatory sequences (promoter, enhancer, silencer, specific sequences for binding further regulatory factors) of the genes coding for the proteins mentioned in table 2.

Moreover, these tools are suitable as means for the molecular definition of inflammatory joint diseases and other inflammatory, infectious or tumorous diseases in the human, thereby making use of the genes, DNA-sequences or the deduced corresponding proteins or peptides mentioned in claims 1 to 3, and the proteins and partial protein sequences from claims 6 to 9 or the respective coding gene sequences.

The tools according to the invention are moreover employed for

- the choice of a therapy for inflammatory joint diseases and other inflammatory, infectious or tumorous diseases in the human, thereby employing the genes, DNA-sequences or deduced corresponding proteins or peptides mentioned in claims 1 to 3,

- the monitoring of the progression/therapeutic success in inflammatory joint diseases and other inflammatory, infectious or tumorous diseases in the human, thereby employing the genes, DNA-sequences or deduced corresponding proteins or peptides mentioned in claims 1 to 3,

- molecular means for the development of therapy concepts, which comprise a direct or indirect impact on the expression of the genes or gene sequences mentioned in claims 1 to 3,

- the development of therapy concepts, which comprise a direct or indirect impact on the expression of the proteins or partial protein sequences mentioned in claims 6 to 9,

- the development of therapy concepts, which comprise a direct or indirect impact on autoreactive T cells being directed against proteins or partial protein sequences mentioned in claims 8 to 11,

- the impact on the biological effect of the proteins deduced from the gene sequences mentioned in claims 1 to 3.

- the impact on the direct molecular regulatory pathways/circuits, in which the genes mentioned in claims 1 to 3 and the proteins deduced thereof are taking part

- the development of therapy concepts with the creation and employment of interpretation algorithms, thereby using the mentioned genes and sequences and their regulatory mechanisms, in order to recognize or predict therapy concepts, therapeutic effects, therapeutic optimizations or disease prognostications

- the development of biologically active drugs (Biologicals) under employment of the genes, gene sequences, regulation of genes or gene sequences, or under employment of proteins, protein sequences, fusion proteins according to claims 1 to 3 and 6 to 9, or under employment of antibodies or autoreactive T cells according to claims 10 to 14.

The use of the claimed tools according to the invention is to be found in the

- analysis of blood samples or tissue samples in medical diagnostics,

- application in analytics according to example 1, and the

- application for therapy concepts according to example 2.

Materials and Methods

Patients and Tissue asservation

All patients were selected according to the ACR-criteria for RA (1) and OA (20). Synovial tissue was immediately transported in RPMI medium (RPMI – conventional cell culture medium, diluting medium RPMI 1640; Moore, G.E. et al., J. Am. Assoc. 199, 519-524, 1967), supplemented with penicillin and streptomycin (100 U/ml each), from the operating room into the laboratory. After the preparation of the synovial membrane, the samples were immediately shock frozen in liquid nitrogen. The samples were stored at -80°C until further use. As samples for the Representational Difference Analysis (RDA), the hybridisation to Unigene filter arrays (http://www.ncbi.nlm.nih.gov/UniGene/) and the hybridisation to Affymetrix arrays, we used synovial tissue samples derived from normal donor (ND), osteoarthrosis (OA) and rheumatoid arthritis (RA).

Isolation of RNA

The samples were homogenized in order to extract RNA: Tissue amounts of <50 mg were crushed to powder by means of mortar and pestle while cooling with liquid nitrogen, followed by the lysis in a guanidine-isothiocyanate containing solution (RLT-buffer from Qiagen, Hilden, Germany - www.qiagen.com/literature/handbooks/rna/rny96/1019545_PREHB_RNY96_prot2.pdf). Larger amounts of tissue were crushed up by means of a tissue homogenizer (IKA-Ultra-Turrax T 25; Jahnke & Kunkel, Staufen) in an icecold, guanidine-isothiocyanate containing solution (RLT-buffer from Qiagen, Hilden, Germany). The isolation of RNA was accomplished by a modified protocol using the phenol-chloroform-extraction according to Chomczynski (21), followed by the immediate isolation of RNA from the aqueous phase by means of the QIAGEN-RNaesy-Kit (see handbook of the manufacturer: http://www.qiagen.com/literature/rnalit.asp#mini). The kit was used according to the manufacturer's protocol. The RNA was eluted in 30-100 µl of RNAse-free water.

For a quality control, the optical density (OD) was measured at 260 nm (OD260), the relation of OD260/OD280nm was determined and a gel electrophoresis was performed on 1% agarose. DNA-contaminations - if necessary - were able to be detected either in the gel or, after the first strand synthesis, in a PCR using an intron-primer for the Glycerol-aldehyde-3-phosphate dehydrogenase (GAPDH). In these exceptional cases we also digested with DNAse, thereby following the instructions of the QIAGEN protocol.

First Strand Synthesis

The cDNA-synthesis was performed under employment of the Superscript II Reverse Transcriptase (RT), including the 5x reaction buffer from Invitrogen/Life Technologies (Karlsruhe, Germany; http://www.invitrogen.com). The employed amounts of RNA were 3-5 µg for the semiquantitative PCR and 10-20 µg for the RDA and the array hybridisations in a final volume of 20 µl. The reaction mix for the transcription into cDNA contained the following components: 500 ng of each respective primer oligonucleotide ($Oligo(dT)_{12-18}$; T7-Oligo (dT_{24})), 50 mM Tris pH 8,3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, deoxynucleotide-triphosphate (dNTP) mixture with each nucleotide in a final concentration of 1 mM, 40 U RNase inhibitor and 20 U Superscript™ II RT. The incubation period was 1,5 hours, followed by the inactivation of the enzymes by heating the samples to 72°C for 15 min.

Second Strand Synthesis

The following components were added to the cDNA by pipetting: 90 µl aqua dest., 30 µl 5x Second strand buffer (500 mM KCl, 50 mM ammonium acetate, 25 mM $MgCl_2$, 0,75 mM beta-nicotinamide-adenine-dinucleotide (β-NAD) and 0,25 mg/ml of bovine serum albumin (BSA)), 3 µl of a 10 mM dNTP-solution and an enzyme solution of the following activities and amounts: 1 µl E. coli ligase (10 U/µl), 4 µl DNA polymerase I (10 U/µl) and 1 µl RNAseH (2U/µl) (Invitrogen/Life Technologies, Karlsruhe, Germany). The incubation period was 2 hours at a temperature of 16°C. After having added 2 µl of a T4 DNA polymerase (5 U/µl), the incubation was pursued for further 30 min at 16°C.

Subtractive Hybridisation and RDA

The PCR Suppression Subtractive Hybridisation (SSH) (22) was performed according to the instructions of the manufacturer of the PCR Select Kit (Clontech, Palo Alto, USA;

http://www.clontech.com/pcr-select/index.shtml). The digest of the double-stranded cDNA was accomplished with the restriction enzyme RsaI from Rhodopseudomonas sphaeroides. For the RDA (18), the double-stranded cDNA was cut with the restriction enzyme DPNII from Diplococcus pneumoniae (20 U in 100 µl). Then, a ligation to adapter primers (RBgl12, RBgl24) was performed, followed by amplification according to published protocols (17, 18). The tester-amplicon was obtained after a further restriction digest with DPNII by means of a ligation to a further adapter oligonucleotide (JBgl12 and JBgl24 or NBgl12 and NBgl24(18)) in the second round of subtraction.

After the hybridisation, the sequences belonging to the tester were selectively amplified by PCR and thereby accumulated in the subtraction product in both methods.

Description of the subtraction samples

The RDA protocols were such modified, that it became possible to identify both genes being expressed in a weaker manner and in a more pronounced manner in the samples derived from RA, OA and normal tissue donors.

In this procedure:

1 OA (driver) was subtracted from RA (tester) in order to obtain sequences, which show a stronger expression in RA- than in OA-tissues

2 RA (driver) was subtracted from ND (tester) in order to obtain sequences, which show a weaker expression in RA-samples than in ND-samples

3 ND (driver) was subtracted from OA (tester) in order to obtain sequences, which show a stronger expression in OA- than in ND-tissues

Performance of the subtraction library cloning, sequence determination and comparison to data bases

The subtraction products of the SSH-sample were cloned into a pCRII vector (TA-Cloning Kit; Invitrogen, Heidelberg, Germany; http://www.invitrogen.com). The subtraction products

from the RDA were cloned into a pBluescript $KS^{+}II$ vector (Stratagene, La Jolla, USA; http://www.stratagene.com/vectors/selection/plasmid1.htm), which had previously been cut with the restriction enzyme BamHI from Bacillus amyloliquefaciens, then being dephosporylated and purified. About 150 clones were isolated and the sequence determined by means of an ABI 377 Sequencer (Applied Biosystems, Weiterstadt, Germany; http://home.appliedbiosystems.com). The sequence determination was performed according to the manufacturer's Dye Terminator Chemistry protocol under employment of a T7-primer.

After the elimination of vector sequences, the comparative analysis of the sequences was performed under employment of the Genebank and NCBI-databases (http://www.ncbi.nlm.nih.gov).

Microarray Hybridisation

Two different chip technologies were used: 1.) Use of filters, onto which the PCR-products of cDNA-clones of the UNIGENE library (http://www.ncbi.nlm.nih.gov/UniGene/) were spotted. The hybridisation was here performed at 65°C with ^{33}P-labelled cDNA-samples after first strand synthesis with oligo($dT_{(12-18)}$) (23, 24). 2.) Hybridisations were performed with the microarrays (HU95A, HU95B, HU95C, HU95D and HU95E) from Affymetrix (Affymetrix Inc., Santa Clara, USA; www.affymetrix.com). These arrays are arrays of oligonucleotides, the base sequences of which are derived from 12.000 known genes and 24.000 Expressed Sequence Tag (EST-) entries. The synthesis of the labeled samples was accomplished according to the manufacturer's technical manual (Affymetrix Inc, Santa Clara, USA).

The fluorescence-labelled sample was synthesized after transcription with an oligo-dT_{24}-primer, which contains a T7 polymerase binding site. The labelling reaction was accomplished under employment of the T7 RNA polymerase and biotinylated dNTPs according to the manufacturer's protocol (ENZO-Biochem, New York, USA; http://www.enzo.com/entrance.html).

In both chip analyses, the sample to be tested and the reference sample were hybridized to separate filters. The comparison of signal intensities was accomplished after normalization.

Evaluation of the chip results – Decision Matrix

The evaluation of the signal intensities was accomplished after normalization by means of the software developed for the respective array and by determining an intensity value for the respective sample according to the Tukey's Biweight Method (http://mathworld.wolfram.com/TukeysBiweight.html).
For the evaluation of the Unigene filter arrays, the algorithm was developed at the Max-Planck-Institute for Molecular Genetics at Berlin-Dahlem (http://algorithms.molgen.mpg.de/).
In case of the chips from Affymetrix, the MicroArraySuite 5.0 Software (http://www.affymetrix.com/products/software/specific/mas.affx) including the manufacturer's standard parameters or preconditions was employed.

For the evaluation of the Affymetrix arrays, the target intensity was set to 100 and the normalization factor to 1 in order to normalize the data, and the scaling factor for each sample was calculated. Chips with comparable scaling factors (factor <4) were included in the comparative analyses. The decision criterion for the detection of a gene (Detection p value) was adjusted at <0,05. The comparative analyses for the respective arrays were performed under the employment of the DMT 3.0 Software from Affymatrix (http://www.affymetrix.com/products/software/specific/dmt.affx).

Thereby, the differences between the perfect matches and the perfect- and mismatch intensities are calculated by means of the Wilcoxon-test (http://faculty.vassar.edu/lowry/wilcoxon.html) and compared to the decision criterion Cut-Off (γ-value <0,04). In the specification of results for comparison of the respective chips, a Change-Call (increased, marginally increased, no change, decreased) and the Signal Log Ratio, a measure for the factor of change, are indicated (factor in logarithmic form).

Decision Criterion

Comparative analyses were in each case performed for all samples (every sample in comparison to every sample of the other group: ND, OA, RA).

In case of the Unigene filter hybridisations, a signal difference of >2 for at least 3 of 4 comparisons, and a detection signal with a p-value <0,01 were taken into account.

The proceeding for the arrays from Affymetrix was as follows: Each RA-sample was compared to each OA-sample both in the direction of an increased and decreased expression. Genes, which in 80% of these comparisons showed a deviation in the sense of "increased" or "decreased" at a regulation factor >2 (signal log ratio >1), were selected as candidate genes. In case of the U95A chip, the selection criterion was determined to be a regulating factor >3.

Semiquantitative PCR

Starting from the detected sequence regions, we selected primers with a comparable annealing temperature and product length. For the primer search, the DNASTAR Primer Select Software (DNASTAR Inc., Madison, USA; http://www.dnastar.com/) was used. Primer synthesis was performed at Gibco-Life Technologies (Karlsruhe, Germany). For the semiquantification of the PCR-products, the real time PCR-System GeneAmp 5700 and the Sybr-Green-PCR-Core Kit (Applied Biosystems, Weiterstedt, Germany; http://europe.appliedbiosystems.com/) were employed.

The amounts of cDNA were coordinated for all samples by means of the real-time amplification results for the GAPDH-specific primers. The quantification of the PCR-products of several further genes was accomplished in relation to the GAPDH-specific product as the internal standard. As a control, β-actin as a second housekeeping gene was amplified and analysed in parallel with all samples.

Gene nomenclature	AccNo.	Primer-localization	Product length (bp)
VDUP1	NM006472	665...684 / 863...840	199
TIMP4	U76456	143...159 / 336...317	194
GPX3	NM002084	424...443 / 528..510	105
ß Actin	X00351-	654...675 / 841...819	188
MMP1	X05231	874...895 / 1080...1057	207
MMP3	X05232	973...996 / 1157...1136	185
LTBP4	M22490	511...534 / 760...737	250
GADD45	M60974	457...475 / 573...557	116
CLU	NM001831	1384...1404 / 1509...1489	126
Ca12	NM001218	930...949 /1 049...1031	196

Immunohistochemistry

A sample of the synovial membrane was used for the histopathological evaluation. Thereby, kryosections having a strength of 6 µm were prepared, air dried and then fixed with a 1:1 mixture of acetone and methanol. The hematoxylin staining was performed according to standard protocols and classified according to histopathological evaluation criteria (25).

Methods and results of the Immunome-analysis

Patterns of autoreactivity on the T cell and B cell level (the "immunome") are determined, which are specific for RA and thus distinguish this disease from other rheumatic or non-rheumatic diseases. The knowledge about the RA-specific immunome is of crucial importance for the development of diagnostic tools, which recognize an arthritic disease much earlier and safer as an RA or show the arthritis not to be an RA, than it is possible nowadays. This again allows to control the RA by suitable drugs before irreversible joint and bone damages have occurred.

For this aim, techniques of Proteomics are employed in order to create tissue specific protein patterns by means of high definition 2D-electrophoresis. These were screened by techniques of Immunomics for known and unknown autoreactivities. Protein spots with a useful sensitivity and specificity are identified by sequencing and MALDI-TOF (26). These proteins are then screened for T cell autoreactivity in the same cohort.

According to the invention, autoreactivity patterns have been established, which are completely specific for RA. In this analysis, it is of a great importance, that no single autoreactivity reveals this specificity. This is only reached by the combination of several autoreactivities. Such patterns, which undoubtedly distinguish a patient with RA from a patient suffering from another rheumatic or non-rheumatic disease, comprise the auto-antigens citrullinated peptides (Cit), IgG, BiP (Heavy Chain Binding Protein), Calpastatin (Calp), RA33 (hnRNP A2) and Calreticulin (Calr). The table shows all possible combinations of five of these autoreactivities (RF, Cit, BiP, RA33 and Calp) and the two possible conditions "positive" and "negative". The highlighted patterns (statistically relevant, $p < 0,01$, Whitney U Test; http://faculty.vassar.edu/lowry/utest.html) are only expressed in RA. Fig. 1 shows the sensitivities for all possible combinations both for RA and the control cohorts. The RA-specific patterns are highlighted in a manner analogous to table 1 and mainly comprise

those, which are fourfold and fivefold positive for the individual parameters. The combination of those autoreactivity profiles, which only occur in RA, yields a specificity of 54%.

Exclusively RA-expressed patterns of the three autoreactivities, which are directed against IgG, Cit and BiP (RF+Cit+BiP+ and RF-Cit+BiP+) yield a total sensitivity of 43%. RA-exclusive patterns of the four autoreactivities, which are directed against IgG, Cit, BiP and RA33 (RF+Cit+Bip+RA33+, RF+Cit+BiP-RA33+ and RF+Cit+BiP+RA33-) show a total sensitivity of 40%. In the analysis of six patterns, a sensitivity of 60% is achieved.

According to first investigations, these patterns are also relevant for patients with early RA. Further candidate antigens, which have already been characterized, comprise the Sa-antigen (5), which probably consists of α-Enolase and citrullinated Vimentin.

The identification of the immunome of RA not only is of diagnostic, but also of pathogenetic relevance. When those T cellular autoreactivities being responsible for driving the early RA are identified, it appears to be possible to develop protocols for therapy, which display a specific effectiveness.

	RF	Citrullin	BiP	Calpastatin	RA33
1					
2					
3					
4					
5	+		+		+
6	+		+		+
7	+	-	-	+	+
8	-	-	+	+	+
9	+	-	+	+	-
10	-	+	+	+	-
11	+	+	-	+	-
12	-	+	-	+	+
13	+	+	+	-	-
14	+	+	-	-	+
15	-	+	+	-	+
16	+	-	+	-	+
17	-	-	+	+	-
18	-	-	-	+	+
19	-	+	-	+	-
20	+			+	-
21	+			-	+
22	-		+	-	+

	RF	Citrullin	BiP	Calpastatin	RA33
23	+				
24		+	+		
25	+				
26		+			+
27	-	-	-	+	-
28	-	-	+	-	-
29	-	-	-	-	+
30	-	+	-	-	-
31	+	-	-	-	-
32					

Scheme 1: Patterns of autoreactivity with RF, Citrullin, BiP, Calpastatin and RA33.

Indicated are all 32 possible quintuple combinations of the autoreactivities directed against IgG (RF), Citrullin, BiP, Calpastatin and RA33. Like in Fig. 1, the RA-specific combinations are highlighted in color.

Advantage

Complex molecular patterns are covered. These patterns can be classified by means of mathematical calculation models into groups and congeniality scales. The respective, derivable classification and knowledge about the association e.g. with the duration of the disease, the clinical disease activity (Disease Activity Score (Ref.)), the inflammatory activity being determined by the increase of the C-reactive Protein or by the sedimentation rate, the radiological joint destruction and the specific influence of drugs, allow to draw the following conclusions from the array-analysis: assignment of the clinical picture to a defined diagnosis and to a subgroup allowing to be molecularly classified, evaluation of the disease activity and the progredience to be expected (prognostic evaluation), perspectives of different therapy forms, recommendation for suitable therapeutic approaches (e.g. Methotrexate instead of Leflunomide, or a combination of Sulfasalazine and Methotrexate instead of Methotrexate alone) and, finally, monitoring of the therapeutic success.

By the employment before and during defined measures of medicinal treatment, it can be determined, which of the employed genes are affected by the drug. It is thereby measured, how the drug affects the gene expression being altered in a disease-typical manner. Starting from this, it can be concluded, which disease-related molecular alterations are still valid in defiance of the therapy. The knowledge about the function of these pathologically active

genes principally allows to elucidate pathophysiological processes of the joint disease and to deduce novel therapy concepts.

Combination of the genes

Table 1:

Accession number	Unigene codification	Name of the Gene	Method	Regulation
X57809	Hs.181125		RDA, Affymetrix	RA>OA
X58141			RDA	RA>OA
X63527	Hs.252723	ribosomal protein L19	RDA	RA>OA
U10362	Hs.75864	chromosome 5 open reading frame 8	RDA	RA>OA
M80244	Hs.184601	NM_003486	RDA	RA>OA
M24594	Hs.20315	interferon-induced protein with tetratricopeptide repeats 1	RDA	OA>RA
U01244	Hs.79732	fibulin 1 isoform C precursor NM_006485	RDA	RA>OA
X02761	Hs.287820	fibronectin 1, isoform 1 preproprotein	RDA	RA>OA , OA>NS
L01124	Hs.165590	ribosomal protein S13	RDA	NS>RA
M65062	Hs.107169	insulin-like growth factor binding protein 5	RDA	
M15330	Hs.126256	interleukin 1, beta		
L13210	Hs.79339	galectin 3 binding protein	RDA	
X05232	Hs.83326	matrix metalloproteinase 3 preproprotein		RA>NS, OA
M22490	Hs.68879	bone morphogenetic protein 4	RDA, Affymetrix	NS>RA
AL034397			RDA	OA>NS
M22806			RDA	OA>NS
X06256	Hs.149609	integrin alpha 5 precursor	Unigene	NS>RA
L49169	Hs.75678	FBJ murine osteosarcoma viral oncogene homolog B	Unigene	NS>RA
AB002409	Hs.57907	small inducible cytokine subfamily A (Cys-Cys), member 21	Unigene	RA>NS
X03473	Hs.226117	H1 histone family, member 0	RDA	OA>NS
M92843	Hs.343586	zinc finger protein 36, C3H type, homolog (mouse)	Unigene	NS>RA
M21121	Hs.241392	small inducible cytokine A5 (RANTES)	Affymetrix	RA>OA

U05259			Affymetrix	RA>OA
U80114	Hs.247987		Affymetrix	RA>OA
U81234	Hs.164021	small inducible cytokine subfamily B (Cys-X-Cys), member 6 (granulocyte chemotac	Affymetrix	RA>OA
D11086	Hs.84	interleukin 2 receptor, gamma chain, precursor	Affymetrix	RA>OA
X97267			Affymetrix	RA>OA
U23852			Affymetrix	RA>OA
AA522530	Hs.111244	RTP801	Affymetrix	RA>OA
AF037335	Hs.5338	carbonic anhydrase XII precursor	Affymetrix	RA>OA
U97145	Hs.19317	GDNF family receptor alpha 2	Affymetrix	RA>OA
AA919102	Hs.95327	CD3D antigen, delta polypeptide (TiT3 complex)	Affymetrix	RA>OA
M63928	Hs.180841	CD27 antigen	Affymetrix	RA>OA
Z49194	Hs.2407	POU domain, class 2, associating factor 1	Affymetrix	RA>OA
AL031983			Affymetrix	RA>OA
D15050	Hs.232068		Affymetrix	RA>OA
X92997	Hs.342651		Affymetrix	RA>OA
J03910			Affymetrix	RA>OA
J04132	Hs.97087	T-cell receptor zeta chain precursor	Affymetrix	RA>OA
M55153	Hs.8265	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	Affymetrix	RA>OA
M12959	Hs.74647		Affymetrix	RA>OA
AF031815	Hs.89230	potassium intermediate	Affymetrix	RA>OA
L31584			Affymetrix	RA>OA
X54489			Affymetrix	RA>OA
AF043129			Affymetrix	RA>OA
X59871	Hs.169294	transcription factor 7 (T-cell specific, HMG-box)	Affymetrix	RA>OA
AI743134	Hs.21858	trinucleotide repeat containing 3	Affymetrix	RA>OA
Y13323	Hs.145296	disintegrin protease	Affymetrix	RA>OA
U77735	Hs.80205	pim-2 oncogene	Affymetrix	RA,OA>NS
U58515	Hs.154138	chitinase 3-like 2	Affymetrix	RA,OA>NS
M17016	Hs.1051	granzyme B precursor	Affymetrix	RA>OA
X03066	Hs.1802	major histocompatibility complex, class II, DO beta	Affymetrix	RA,OA>NS
M28170	Hs.96023	CD19 antigen	Affymetrix	RA,OA>NS
L24564	Hs.1027	Ras-related associated with diabetes	Affymetrix	NS>RA
M68840	Hs.183109	monoamine oxidase A	Affymetrix	NS>RA
U76456	Hs.190787	tissue inhibitor of metalloproteinase 4	Affymetrix	OA>RA

		metalloproteinase 4 precursor		
D13814	Hs.89472	angiotensin receptor 1 NM_004835	Affymetrix	NS>RA
AA420624	Hs.183109	monoamine oxidase A	Affymetrix	OA>RA
X51757	Hs.3268	heat shock 70kD protein 6 (HSP70B')	Affymetrix	NS>RA
U29344	Hs.83190	fatty acid synthase	Affymetrix	NS>RA
L19871	Hs.460	activating transcription factor 3 long isoform NM_004024	Affymetrix	NS>RA
J02611	Hs.75736	apolipoprotein D precursor	Affymetrix	NS>RA
M12272	Hs.2523	class I alcohol dehydrogenase, gamma subunit	Affymetrix	NS>RA
L34041	Hs.348601	glycerol-3-phosphate dehydrogenase 1 (soluble)	Affymetrix	NS>RA
L12760	Hs.1872	phosphoenolpyruvate carboxykinase 1 (soluble)	Affymetrix	OA>RA
M63978			Affymetrix	RA>OA
S95936	Hs.284176	transferrin precursor	Affymetrix	NS>RA
U42031	Hs.7557	FK506-binding protein 5	Affymetrix	NS>RA
Z97171			Affymetrix	NS>RA
S69790			Affymetrix	NS>RA
U41843	Hs.295362	DR1-associated protein 1 (negative cofactor 2 alpha)	Affymetrix	OA,NS>RA
AL049653			Affymetrix	NS>RA
M31682	Hs.1735	inhibin beta B subunit precursor	Affymetrix	NS>RA
AF009767	Hs.132898	fatty acid desaturase 1	Affymetrix	NS>RA,OA
X02910	Hs.241570	tumor necrosis factor (cachectin)		
AB023152	Hs.12183		Affymetrix	NS>RA,OA
U37283	Hs.300946	Microfibril-associated glycoprotein-2	Affymetrix	OA,NS>RA
X05451	Hs.158295		Affymetrix	OA,NS>RA
W26480	Hs.132898	fatty acid desaturase 1	Affymetrix	NS>RA
D14874	Hs.394	adrenomedullin	Affymetrix	RA>NS
M12174	Hs.204354	ras homolog gene family, member B	Affymetrix	NS>RA
M60974	Hs.80409	growth arrest and DNA-damage-inducible, alpha	Affymetrix	NS>RA
S62138			Affymetrix	NS>RA
X16706	Hs.301612	FOS-like antigen 2	Affymetrix	NS>RA
X56667	Hs.106857	calbindin 2, full length protein isoform NM_007087	Affymetrix	NS>RA
H15814			Affymetrix	NS>RA
AL021977			Affymetrix	NS>RA
U80055			Affymetrix	NS>RA

U09564	Hs.75761	SFRS protein kinase 1	Affymetrix	RA>OA
U14407	Hs.168132	interleukin 15	Affymetrix	RA>OA
U27185	Hs.82547	retinoic acid receptor responder (tazarotene induced) 1	Affymetrix	RA>OA
Z35278	Hs.170019	runt-related transcription factor 3	Affymetrix	RA>OA
M12886	Hs.303157		Affymetrix	RA>OA
L05424			Affymetrix	RA>OA
L09230	Hs.301921	chemokine (C-C motif) receptor 1	Affymetrix	RA>OA
L22075	Hs.1666	guanine nucleotide binding protein (G protein), alpha 13	Affymetrix	RA>OA
M28130			Affymetrix	RA>OA
M29696	Hs.237868	interleukin 7 receptor	Affymetrix	RA>OA
M31165	Hs.29352	tumor necrosis factor, alpha-induced protein 6	Affymetrix	RA>OA
M16038	Hs.80887	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	Affymetrix	RA>OA
X83490			Affymetrix	RA>OA
D13666	Hs.136348	osteoblast specific factor 2 (fasciclin I-like)	Affymetrix	RA>OA
L10717	Hs.211576	IL2-inducible T-cell kinase	Affymetrix	RA>OA
X04500	Hs.126256	interleukin 1, beta	Affymetrix	RA>OA
U24153	Hs.30692	p21 (CDKN1A)-activated kinase 2	Affymetrix	RA>OA
M32315	Hs.256278	tumor necrosis factor receptor 2 (75kD)	Affymetrix	RA>OA
U51903	Hs.78993	IQ motif containing GTPase activating protein 2	Affymetrix	RA>OA
AF002700	Hs.19317	GDNF family receptor alpha 2	Affymetrix	RA>OA
U37518	Hs.83429	tumor necrosis factor (ligand) superfamily, member 10	Affymetrix	RA>OA
HG1103-HT1103			Affymetrix	RA>OA
HG3521-HT3715			Affymetrix	RA>OA
AF024710			Affymetrix	RA>OA
U01134	Hs.138671	fms-related tyrosine kinase 1 (vascular endothelial growth factor	Affymetrix	RA>OA
U27467	Hs.227817	BCL2-related protein A1	Affymetrix	RA>OA
M79321	Hs.80887	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	Affymetrix	RA>OA

J04765	Hs.313	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte	Affymetrix	RA>OA
M21154	Hs.262476	S-adenosylmethionine decarboxylase 1 precursor	Affymetrix	RA>OA
AF098641	Hs.306278		Affymetrix	RA>OA
D63789	Hs.174228	small inducible cytokine subfamily C, member 2	Affymetrix	RA>OA
S68134	Hs.351252	cAMP responsive element modulator	Affymetrix	RA>OA
AB014515	Hs.323712	KIAA0615 gene product	Affymetrix	RA>OA
AI800499	Hs.161002		Affymetrix	RA>OA
Y13710	Hs.16530	small inducible cytokine subfamily A (Cys-Cys), member 18, pulmonary and activat	Affymetrix	RA>OA
AJ011915	Hs.184376	synaptosomal-associated protein, 23kD	Affymetrix	RA>OA
AF030339	Hs.286229	plexin C1	Affymetrix	RA>OA
X17042	Hs.1908	proteoglycan 1, secretory granule	Affymetrix	RA>OA
AF059214	Hs.194687	cholesterol 25-hydroxylase	Affymetrix	RA>OA
D42043	Hs.79123		Affymetrix	RA>OA
M24283	Hs.168383	intercellular adhesion molecule 1 precursor	Affymetrix	RA>OA
AF042729	Hs.171776	inositol(myo)-1(or 4)-monophosphatase 1	Affymetrix	RA>OA
M64595	Hs.173466	ras-related C3 botulinum toxin substrate 2	Affymetrix	RA>OA
AA868382	Hs.198253	major histocompatibility complex, class II, DQ alpha 1	Affymetrix	RA>OA
AB006746	Hs.198282	phospholipid scramblase 1	Affymetrix	RA>OA
X00437	Hs.303157		Affymetrix	RA>OA
M59287			Affymetrix	RA>OA
AA725102	Hs.51305	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	Affymetrix	RA>OA
M97935	Hs.21486	signal transducer and activator of transcription 1, 91kD	Affymetrix	RA>OA
X54134	Hs.31137	protein tyrosine phosphatase, receptor type, E	Affymetrix	RA>OA
U89942	Hs.83354	lysyl oxidase-like 2	Affymetrix	RA>OA
AF099935	Hs.17839	TNF-induced protein	Affymetrix	RA>OA
M93056			Affymetrix	RA>OA
M97936			Affymetrix	RA>OA

AI887421	Hs.82547	retinoic acid receptor responder (tazarotene induced) 1	Affymetrix	RA>OA
D50532	Hs.54403	macrophage lectin 2 (calcium dependent)	Affymetrix	RA>OA
AI813532	Hs.256278	tumor necrosis factor receptor 2 (75kD)	Affymetrix	RA>OA
U02020	Hs.239138	pre-B-cell colony-enhancing factor	Affymetrix	RA>OA
X05276	Hs.250641	tropomyosin 4	Affymetrix	RA>OA
AF006516	Hs.24752	spectrin SH3 domain binding protein 1	Affymetrix	RA>OA
AB018301	Hs.22039		Affymetrix	RA>OA
AB010812	Hs.22900	nuclear factor (erythroid-derived 2)-like 3	Affymetrix	RA>OA
AF052124	Hs.313	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte	Affymetrix	RA>OA
AB008775	Hs.104624	aquaporin 9	Affymetrix	RA>OA
AF024714	Hs.105115	absent in melanoma 2	Affymetrix	RA>OA
M28696	Hs.278443	Fc fragment of IgG, low affinity IIb, receptor for (CD32)	Affymetrix	RA>OA
X62573			Affymetrix	RA>OA
X07834	Hs.318885	superoxide dismutase 2, mitochondrial	Affymetrix	RA>OA
AL050267	Hs.23889	DKFZP564A032 protein	Affymetrix	RA>OA
U83461	Hs.24030	solute carrier family 31 (copper transporters), member 2	Affymetrix	RA>OA
AB018285	Hs.321707		Affymetrix	RA>OA
AF007875	Hs.5085	dolichyl-phosphate mannosyltransferase polypeptide 1	Affymetrix	RA>OA
X78686	Hs.89714	small inducible cytokine subfamily B (Cys-X-Cys), member 5 (epithelial-derived n	Affymetrix	RA>OA
AF053712	Hs.115770		Affymetrix	RA>OA
AF006083	Hs.5321	ARP3 actin-related protein 3 homolog	Affymetrix	RA>OA
AL050025	Hs.5344	adaptor-related protein complex 1, gamma 1 subunit	Affymetrix	RA>OA
M17017	Hs.624	interleukin 8	Affymetrix	RA>OA
AI651024	Hs.15780		Affymetrix	RA>OA
AF038172			Affymetrix	RA>OA
M55542	Hs.62661	guanylate binding protein 1, interferon-inducible, 67kD	Affymetrix	RA>OA

U11276	Hs.169824	killer cell lectin-like receptor subfamily B, member 1	Affymetrix	RA>OA
Z19585	Hs.75774	thrombospondin 4	Affymetrix	OA>RA
L27560			Affymetrix	OA>RA
M98539			Affymetrix	OA>RA
J00153			Affymetrix	OA>RA
M25079	Hs.155376	hemoglobin, beta	Affymetrix	OA>RA
M80482	Hs.170414	paired basic amino acid cleaving system 4	Affymetrix	OA>RA
L48215	Hs.155376	hemoglobin, beta	Affymetrix	OA>RA
AA524547	Hs.160318	phospholemman, isoform b precursor NM_005031	Affymetrix	OA>RA
AL038340			Affymetrix	OA>RA
AI381790	Hs.74120	adipose specific 2	Affymetrix	OA>RA
X00129	Hs.76461	retinol-binding protein 4, plasma precursor	Affymetrix	OA>RA
U66619	Hs.71622	SWI	Affymetrix	OA>RA
M30038	Hs.334455	alpha tryptase I precursor	Affymetrix	OA>RA
U13666	Hs.184907	G protein-coupled receptor 1	Affymetrix	OA>RA
L05144	Hs.1872	phosphoenolpyruvate carboxykinase 1 (soluble)	Affymetrix	OA>RA
U39447	Hs.198241	copper containing amine oxidase 3 precursor	Affymetrix	OA>RA
AL049313			Affymetrix	OA>RA
AL050125			Affymetrix	OA>RA
D12485			Affymetrix	OA>RA
X78416	Hs.3155	casein, alpha	Affymetrix	OA>RA
AB028998	Hs.6147		Affymetrix	OA>RA
AB020629	Hs.38095	ATP-binding cassette, sub-family A member 8	Affymetrix	OA>RA
X03350	Hs.4	class I alcohol dehydrogenase, beta subunit	Affymetrix	OA>RA
AJ224677	Hs.7122	scrapie responsive protein 1	Affymetrix	OA>RA
AB018317	Hs.22201		Affymetrix	OA>RA
AF009314			Affymetrix	OA>RA
L77730			Affymetrix	OA>RA
D76435	Hs.41154	Zic family member 1 (odd-paired homolog, Drosophila)	Affymetrix	OA>RA
W28828	Hs.133988		Affymetrix	OA>RA
M73720			Affymetrix	OA>RA
M55150	Hs.73875	fumarylacetoacetase	Affymetrix	OA>RA
U13616	Hs.75893	ankyrin 3, isoform 2 NM_020987	Affymetrix	OA>RA
AB005293	Hs.103253	perilipin	Affymetrix	OA>RA
L07765	Hs.76688	carboxylesterase 1 (monocyte	Affymetrix	OA>RA

X82209	Hs.268515	meningioma 1	Affymetrix	OA>RA
J03507	Hs.78065	complement component 7 precursor	Affymetrix	OA>RA
AF013570	Hs.78344	smooth muscle myosin heavy chain 11, isoform SM1 NM_022870	Affymetrix	OA>RA
U70370	Hs.84136	paired-like homeodomain transcription factor 1	Affymetrix	OA>RA
U75744	Hs.88646	deoxyribonuclease I-like 3	Affymetrix	OA>RA
M60278	Hs.799	diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth f	Affymetrix	OA>RA
AF042166	Hs.81008	filamin B, beta (actin binding protein 278)	Affymetrix	OA>RA
J00123			Affymetrix	OA>RA
AI207842	Hs.8272	prostaglandin D2 synthase (21kD, brain)	Affymetrix	OA>RA
AA128249	Hs.83213	fatty acid binding protein 4, adipocyte	Affymetrix	OA>RA
AA152406	Hs.114346	cytochrome c oxidase subunit VIIa polypeptide 1 (muscle) precursor	Affymetrix	OA>RA
AF093118	Hs.11494	fibulin 5	Affymetrix	OA>RA
L38486	Hs.296049		Affymetrix	OA>RA
U66689			Affymetrix	OA>RA
AF049884	Hs.350266	Arg	Affymetrix	OA>RA
AB011089	Hs.12372	tripartite motif protein TRIM2	Affymetrix	OA>RA
AF060568			Affymetrix	OA>RA
AF059293	Hs.114948	cytokine receptor-like factor 1	Affymetrix	OA>RA
AC003107	Hs.1584	cartilage oligomeric matrix protein presursor	Affymetrix	OA>RA
J05037	Hs.76751	serine dehydratase	Affymetrix	OA>RA
D45371	Hs.80485	adipose most abundant gene transcript 1	Affymetrix	OA>RA
U78190			Affymetrix	OA>RA
U24578	Hs.444	serine	Affymetrix	OA>RA
M15856	Hs.180878	lipoprotein lipase precursor	Affymetrix	OA>RA
AF055033	Hs.107169	insulin-like growth factor binding protein 5	Affymetrix	OA>RA
AA976838	Hs.268571	apolipoprotein C-I precursor	Affymetrix	OA>RA
L13698	Hs.65029	growth arrest-specific 1	Affymetrix	OA>RA
AB020316	Hs.134015	uronyl-2-sulfotransferase	Affymetrix	OA>RA
U32324	Hs.64310	interleukin 11 receptor, alpha	Affymetrix	OA>RA
S67070	Hs.78846	heat shock 27kD protein 2	Affymetrix	OA>RA
M12529	Hs.169401	apolipoprotein E	Affymetrix	OA>RA

D50495	Hs.80598	transcription elongation factor A (SII), 2	Affymetrix	OA>RA
D00632	Hs.336920	plasma glutathione peroxidase 3 precursor	Affymetrix	OA>RA
AI760613	Hs.29283		Affymetrix	RA>OA
AW014646	Hs.303157		Affymetrix	RA>OA
W74027	Hs.132906	19A24 protein	Affymetrix	RA>OA
W72338	Hs.23703		Affymetrix	RA>OA
AI805006	Hs.8882		Affymetrix	RA>OA
W67655			Affymetrix	RA>OA
AA631460	Hs.285814		Affymetrix	RA>OA
AI741321	Hs.10760	asporin (LRR class 1)	Affymetrix	RA>OA
AI983115	Hs.132781	class I cytokine receptor	Affymetrix	RA>OA
AI535730	Hs.262958		Affymetrix	RA>OA
AA977937	Hs.102308	potassium inwardly-rectifying channel, subfamily J, member 8	Affymetrix	RA>OA
AA447232	Hs.334838		Affymetrix	RA>OA
AI720806	Hs.49943		Affymetrix	RA>OA
W23237	Hs.296162		Affymetrix	RA>OA
AI762695	Hs.146381	RNA binding motif protein, X chromosome	Affymetrix	RA>OA
AI653211	Hs.96657		Affymetrix	RA>OA
AA633405	Hs.1101	POU domain, class 2, transcription factor 2	Affymetrix	RA>OA
N78018	Hs.267566	hypothetical protein FLJ20371	Affymetrix	RA>OA
AI625959	Hs.112242		Affymetrix	RA>OA
T66196	Hs.111554	ADP-ribosylation factor-like 7	Affymetrix	RA>OA
AI697841	Hs.20450	BCM-like membrane protein precursor NM_014036	Affymetrix	RA>OA
AA569128	Hs.283021	chloride intracellular channel 5	Affymetrix	OA>RA
R53594	Hs.260164		Affymetrix	OA>RA
AI970898	Hs.234898		Affymetrix	OA>RA
AI972390	Hs.348493		Affymetrix	OA>RA
N23769	Hs.26691		Affymetrix	OA>RA
AI806324	Hs.28625		Affymetrix	OA>RA
N28741	Hs.75354		Affymetrix	OA>RA
AL040912	Hs.31595	oligodendrocyte transmembrane protein	Affymetrix	OA>RA
AI681917	Hs.3321		Affymetrix	OA>RA
AW006235	Hs.41502	hypothetical protein FLJ21276	Affymetrix	OA>RA
W73819	Hs.352100		Affymetrix	OA>RA
T77033	Hs.182364		Affymetrix	OA>RA
AW015787	Hs.237731		Affymetrix	OA>RA
N30858	Hs.44234	triggering receptor expressed on myeloid	Affymetrix	OA>RA

		cells 2		
AI810669	Hs.44829		Affymetrix	OA>RA
N49922	Hs.1787	proteolipid protein1 (Pelizaeus-Merzbacher disease, spastic paraplegia 2, uncomp	Affymetrix	OA>RA
AA082546	Hs.48516		Affymetrix	OA>RA
AI694320	Hs.6295		Affymetrix	OA>RA
AI632283	Hs.47448		Affymetrix	OA>RA
AA039324	Hs.201925		Affymetrix	OA>RA
AA877186	Hs.90250		Affymetrix	OA>RA
R42166	Hs.94000		Affymetrix	OA>RA
AI631882	Hs.6510	thyrotropin-releasing hormone degrading ectoenzyme	Affymetrix	OA>RA
W68636	Hs.168640	ankylosis, progressive homolog NM_054027 ankylosis, progressive homolog	Affymetrix	OA>RA
AA700227	Hs.10119		Affymetrix	OA>RA
AI948584	Hs.350495		Affymetrix	OA>RA
AI678080	Hs.141693		Affymetrix	OA>RA
AI732274	Hs.11006		Affymetrix	OA>RA
AI341383	Hs.349764		Affymetrix	OA>RA
Z99386	Hs.173638		Affymetrix	OA>RA
W95023	Hs.173933		Affymetrix	OA>RA
AI860775	Hs.98506		Affymetrix	OA>RA
AA464846	Hs.103262		Affymetrix	OA>RA
AI751698	Hs.184907	G protein-coupled receptor 1	Affymetrix	OA>RA
AA545730	Hs.293821		Affymetrix	OA>RA
AA181060	Hs.349283		Affymetrix	OA>RA
AA195184			Affymetrix	OA>RA
AI680541	Hs.23767	hypothetical protein FLJ12666	Affymetrix	OA>RA
AI659533	Hs.348490		Affymetrix	OA>RA
AI750575	Hs.173933		Affymetrix	OA>RA
AI870335	Hs.32450		Affymetrix	OA>RA
AA160945	Hs.14479		Affymetrix	OA>RA
AI936699	Hs.193784		Affymetrix	OA>RA
AI130027	Hs.293539		Affymetrix	OA>RA
AA081093	Hs.68055		Affymetrix	OA>RA
AA142913	Hs.71721		Affymetrix	OA>RA
AI984000	Hs.37482	COPZ2 for nonclathrin coat protein zeta-COP	Affymetrix	OA>RA
AI864898	Hs.43125		Affymetrix	OA>RA
AI670876	Hs.44276	homeo box C10	Affymetrix	OA>RA
AA541787	Hs.23837		Affymetrix	OA>RA
AA775711	Hs.348392		Affymetrix	OA>RA
AI659927	Hs.6634		Affymetrix	OA>RA
AI084224	Hs.53542		Affymetrix	OA>RA

AI123555	Hs.81796		Affymetrix	OA>RA
W73230	Hs.7913		Affymetrix	OA>RA
W27376	Hs.8395	hypothetical protein FLJ10781	Affymetrix	OA>RA
AW022607	Hs.12482	glyceronephosphate O-acyltransferase	Affymetrix	OA>RA
W70242	Hs.58086		Affymetrix	OA>RA
W25528	Hs.89319		Affymetrix	OA>RA
AA947123	Hs.8861		Affymetrix	OA>RA
AA528821	Hs.235857		Affymetrix	OA>RA
AA131648	Hs.23767	hypothetical protein FLJ12666	Affymetrix	OA>RA
R12398	Hs.21075	GTF2I repeat domain-containing 1, isoform 1 NM_005685	Affymetrix	OA>RA
W52683	Hs.107260	hypothetical protein DKFZp586H0623	Affymetrix	OA>RA
W72194	Hs.108924	ponsin NM_015385	Affymetrix	OA>RA
AA885516	Hs.104627		Affymetrix	OA>RA
W68796	Hs.237731		Affymetrix	OA>RA
AI879337	Hs.323432	mammalian inositol hexakisphosphate kinase 2	Affymetrix	OA>RA
W45581	Hs.23133		Affymetrix	OA>RA
N98637	Hs.7759		Affymetrix	OA>RA
AI809953	Hs.123933		Affymetrix	OA>RA
T68423	Hs.11873		Affymetrix	OA>RA
AL044670	Hs.182364		Affymetrix	OA>RA
AA779895	Hs.19339		Affymetrix	OA>RA
AI719167	Hs.12731		Affymetrix	OA>RA
T99215	Hs.168640	ankylosis, progressive homolog NM_054027 ankylosis, progressive homolog	Affymetrix	OA>RA
AA534296	Hs.20953		Affymetrix	OA>RA
AI819043	Hs.21342		Affymetrix	OA>RA
AI762879	Hs.86437		Affymetrix	RA>OA
W61000	Hs.238730		Affymetrix	RA>OA
AL043192	Hs.103378		Affymetrix	RA>OA
AI741313	Hs.103657		Affymetrix	RA>OA
AI031674	Hs.236494	ras-related GTP-binding protein	Affymetrix	RA>OA
AA670193			Affymetrix	RA>OA
AW005250	Hs.238936		Affymetrix	RA>OA
AA682496	Hs.270737	tumor necrosis factor (ligand) superfamily, member 13b	Affymetrix	RA>OA
AI128225	Hs.914		Affymetrix	RA>OA
AW026543	Hs.238936		Affymetrix	RA>OA
AI991095	Hs.293441		Affymetrix	RA>OA
AI872510	Hs.181125		Affymetrix	RA>OA
AI828404	Hs.300697		Affymetrix	RA>OA

AI807353	Hs.237868	interleukin 7 receptor	Affymetrix	RA>OA
AL048481	Hs.11571		Affymetrix	RA>OA
AW014626	Hs.10949		Affymetrix	RA>OA
AI400414			Affymetrix	RA>OA
AI655112	Hs.16179	hypothetical protein FLJ23467	Affymetrix	RA>OA
AI936345	Hs.95549	hypothetical protein	Affymetrix	RA>OA
AI961907	Hs.179573	alpha 2 type I collagen preproprotein	Affymetrix	RA>OA
AI743730	Hs.30822	hypothetical protein FLJ11110	Affymetrix	RA>OA
AI990512	Hs.34192		Affymetrix	RA>OA
AI741715	Hs.1466	glycerol kinase	Affymetrix	RA>OA
T66305	Hs.12920	hypothetical protein FLJ20668	Affymetrix	RA>OA
AA424160	Hs.165909		Affymetrix	RA>OA
AI075407	Hs.296083		Affymetrix	RA>OA
AA811088	Hs.24143	WASP-interacting protein	Affymetrix	RA>OA
AI978918	Hs.179608	retinol dehydrogenase homolog	Affymetrix	RA>OA
AA740831	Hs.193514		Affymetrix	RA>OA
W84421	Hs.349096		Affymetrix	RA>OA
AA233208	Hs.91165	hypothetical protein	Affymetrix	RA>OA
AA886976	Hs.95821	osteoclast stimulating factor 1	Affymetrix	RA>OA
AA864400	Hs.71215	docking protein 2, 56kD	Affymetrix	RA>OA
AI073984	Hs.14453	interferon consensus sequence binding protein 1	Affymetrix	RA>OA
AI983633	Hs.179573	alpha 2 type I collagen preproprotein	Affymetrix	RA>OA
AI564488	Hs.300697		Affymetrix	RA>OA
AI655781	Hs.237868	interleukin 7 receptor	Affymetrix	RA>OA
AA814195	Hs.184465	hypothetical protein FLJ11259	Affymetrix	RA>OA
AI916783	Hs.234149	hypothetical protein FLJ20647	Affymetrix	RA>OA
AA829355	Hs.267993	hypothetical protein FLJ10143	Affymetrix	RA>OA
N66595	Hs.24283		Affymetrix	RA>OA
AA165400	Hs.10927		Affymetrix	RA>OA
AI478759	Hs.234149	hypothetical protein FLJ20647	Affymetrix	RA>OA
AI655719	Hs.2157	Wiskott-Aldrich syndrome protein	Affymetrix	RA>OA
N63815	Hs.110121	SEC7 homolog	Affymetrix	RA>OA
AW001184	Hs.44672	hypothetical protein FLJ10470	Affymetrix	RA>OA
N21390	Hs.5888		Affymetrix	RA>OA
AA587944	Hs.259737	FN5 protein	Affymetrix	RA>OA
AI951459	Hs.7337	hypothetical protein FLJ10936	Affymetrix	RA>OA

		FLJ10936		
AA464464	Hs.10949		Affymetrix	RA>OA
AI692538	Hs.11135		Affymetrix	RA>OA
AI817147	Hs.181301	cathepsin S	Affymetrix	RA>OA
AI263085	Hs.17914	CD20-like precusor	Affymetrix	RA>OA
W58252	Hs.182793	golgi phosphoprotein 2	Affymetrix	RA>OA
AA056180	Hs.70704		Affymetrix	RA>OA
AA224174	Hs.111099		Affymetrix	OA>RA
AI571452	Hs.11169	Gene 33	Affymetrix	OA>RA
AA155952	Hs.349303		Affymetrix	OA>RA
W68504	Hs.191098		Affymetrix	OA>RA
AI200456	Hs.48516		Affymetrix	OA>RA
AW003093	Hs.349764		Affymetrix	OA>RA
AI190027	Hs.38034		Affymetrix	OA>RA
R52934	Hs.8562	hypothetical protein FLJ20374	Affymetrix	OA>RA
W44633	Hs.301296		Affymetrix	OA>RA
AW024474	Hs.44276	homeo box C10	Affymetrix	OA>RA
AI806502	Hs.334800		Affymetrix	OA>RA
AI492370	Hs.105606	hypothetical protein FLJ20512	Affymetrix	OA>RA
AW021179	Hs.90443	NADH dehydrogenase (ubiquinone) Fe-S protein 8 (23kD) (NADH-coenzyme Q reductase	Affymetrix	OA>RA
AI679110	Hs.323067		Affymetrix	OA>RA
R85633			Affymetrix	OA>RA
N91161	Hs.117176	poly(A)-binding protein, nuclear 1	Affymetrix	OA>RA
AW020657			Affymetrix	OA>RA
AI871043	Hs.173233	hypothetical protein FLJ10970	Affymetrix	OA>RA
N39237	Hs.44977		Affymetrix	OA>RA
AI949833	Hs.21914		Affymetrix	OA>RA
AA679297	Hs.109494	secreted protein of unknown function	Affymetrix	OA>RA
AI962647	Hs.182364		Affymetrix	OA>RA
AL037611	Hs.285902		Affymetrix	OA>RA
AI871278	Hs.301804		Affymetrix	OA>RA
AI357650	Hs.28847	AD026 protein	Affymetrix	OA>RA
AI149793	Hs.38034		Affymetrix	OA>RA
AI797684	Hs.39619	hypothetical protein LOC57333	Affymetrix	OA>RA
R52250	Hs.348297		Affymetrix	OA>RA
AI669738	Hs.128856	CSR1 protein	Affymetrix	OA>RA
AA058770	Hs.18987		Affymetrix	OA>RA
AI039005	Hs.164680		Affymetrix	OA>RA
AI936560	Hs.6136		Affymetrix	OA>RA
AA521373	Hs.9469	pleckstrin homology domain-containing, family A (phosphoinositide	Affymetrix	OA>RA

binding specif

H15888	Hs.27621	sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembran	Affymetrix	OA>RA
AI333793	Hs.337062		Affymetrix	OA>RA
AA523172	Hs.103135		Affymetrix	OA>RA
AI860960	Hs.352081		Affymetrix	OA>RA
AI355848	Hs.35841	nuclear factor I	Affymetrix	OA>RA
AI982754	Hs.75106	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testos	Affymetrix	OA>RA
AI800218	Hs.289019	latent transforming growth factor beta binding protein 3	Affymetrix	OA>RA
AW016356	Hs.126857		Affymetrix	OA>RA
AA968552	Hs.25523		Affymetrix	OA>RA
AI634557	Hs.28107		Affymetrix	OA>RA
AW025494	Hs.95867	hypothetical protein EST00098	Affymetrix	OA>RA
AA628405	Hs.339352		Affymetrix	OA>RA
AI810399	Hs.55940		Affymetrix	OA>RA
AA029735	Hs.159993		Affymetrix	OA>RA
AA723927	Hs.209569		Affymetrix	OA>RA
AI799784	Hs.49696		Affymetrix	OA>RA
AI817330	Hs.110477	dolichyl-phosphate mannosyltransferase polypeptide 3	Affymetrix	OA>RA
AI990803	Hs.293782		Affymetrix	OA>RA
AA034418	Hs.30627		Affymetrix	OA>RA
AA115295	Hs.284208	DKFZP434N161 protein	Affymetrix	OA>RA
AI673281	Hs.181444	hypothetical protein	Affymetrix	OA>RA
W63805	Hs.84344	CGI-135 protein	Affymetrix	OA>RA
AA427597		TGFß-induc early growth response 2	Unigene	NS>RA
AA806239		IG-ALPHA2-C REGION	Unigene	RA>NS
AB014518		KIAA0618	Unigene	RA>NS
AB021871		AK1	RDA, Unigene	RA>OA, RA>NS
AF 000984		DBY altern transcript 2	Affymetrix	NS>RA
AF 001691		cornified envelope precursor	Affymetrix	NS>RA
AF005058		CXC		
AF0605668		leukemia zink finger PLZF	Affymetrix	OA>RA
AF068293		HDCMB07P/PCM-1	Unigene	RA>NS
AF105036		GKLF	RDA	OA>NS
AF182035		a Actin	RDA	OA>NS

AF182035	myosin light chain	RDA	OA>NS
AF216292	BIP		
AF218004	CSNK1A1	Unigene	RA>NS
AJ000542	natural killer cell receptor p58	RDA	RA>OA
J05008	EDN1	Affymetrix	NS>RA
L08187	cytokine receptor EBI 3	RDA	RA>OA
L31581	EBI1/CCR7	Affy	RA>NS
L37036	ENA-78	=Affymetrix	RA>OA
M10988	TNFμ		
M19997	elongation factor 2	RDA	RA>OA
M29469	Ig rearranged k chain (VJ regions)	RDA, Affymetrix	RA>OA
M31164	TSG6	RDA, Unigene	RA>OA, RA>NS
M83248	OSTP (Osteopontin)	RDA, Affymetrix	RA>OA
NM_002450	Metallomethionein	Unigene	NS>RA
NM_003573	TGFß-BP4	Unigene	RA>NS
NM_000362	TIMP-3	RDA	NS>RA
NM_000396	Cathepsin K	RDA	RA>OA , OA>NS
NM_0006091	SDF1	RDA	OA>NS
NM_001908	Cathepsin B	RDA	OA>NS
NM_002084	glutathion peroxidase 3	RDA	NS>RA
NM_002229	Jun B	Unigene	NS>RA
NM_002989	SLC	Unigene	RA>NS
NM_003966	SEMA5A	RDA	RA>OA
NM_004039	Annexin II	RDA	RA>OA , OA>NS
NM_005368	Myoglobin	RDA	OA>NS
NM_006472	VDUP1	RDA, Unigene	NS>RA
NM_007016	Mysin light polypeptid2	RDA	OA>NS
NM_015675	GADD45B/MYD118	RDA, Unigene	NS>RA
R75775	EGR1	Unigene	NS>RA
U070136	megakaryocyte stimulating factor	RDA, Unigene	NS>RA
U34690	CORO1A/ p57	Unigene	RA>NS
U93569	L1 element	RDA, Unigene	RA>OA; RA>NS
X03754	SCYA3 (MIP a) /GOS19	Unigene	RA>NS
X0523	MMP1		
X14723	Clustrin / SP40	RDA, Unigene	NS>RA
X15332	collagen III a1	RDA, Unigene	RA>OA
X54629	c-myc	RDA, Unigene	NS>RA
X54629	pHL-1 gene	RDA	NS>RA
X58122	Nebulin	RDA	OA>NS
X62996	mitochondrial mRNA	RDA	OA>NS
X63596	TRE-2	RDA	RA>OA
X65968	PMP22	Unigene	RA>NS
X88971	HLA DRB1	RDA	RA>OA

X94771	EMP3	Unigene	RA>NS
XM 008868	latent transforming growth factor beta binding prot. LTBP4	RDA, Unigene	NS>RA
XM_031289	interleukin 8	=Affymetrix	RA>OA
XM012651	collagen I a1	RDA	RA>OA

Combination of the proteins

Table 2:

Proteine	**Example for accession**
78 kDa glucose-regulated protein precursor (GRP 78) (Immunoglobulin heavy chain binding protein) (BIP) (Endoplasmic reticulum lumenal Ca2+ binding protein grp78)	P11021
Citrullinierte Peptide (Peptids containing the deiminated form of Arginin [Citrullin])	
Sa-Antigen	
RA33 / Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2 / hnRNP B1)	P22626
Calpain inhibitor (Calpastatin) (Sperm BS-17 component)	P20810
Calreticulin precursor (CRP55) (Calregulin) (HACBP) (ERp60)	P27797
Synovial stimulatory protein P205	P80697
Filaggrin precursor	P20930
Fibrin	
Fibrinogen alpha/alpha-E chain precursor [Contains: Fibrinopeptide A]	P02671
Fibrinogen beta chain precursor [Contains: Fibrinopeptide B]	P02675
Fibrinogen gamma chain precursor (PRO2061)	P02679
DnaJ	
Ig gamma-1 chain C region	P01857
Ig gamma-2 chain C region	P01859
Ig gamma-3 chain C region (Heavy chain disease protein) (HDC)	P01860
Ig gamma-4 chain C region	P01861
60 kDa heat shock protein, mitochondrial precursor (Hsp60) (60 kDa chaperonin) (CPN60) (Heat shock protein 60) (HSP-60) (Mitochondrial matrix protein P1) (P60 lymphocyte protein) (HuCHA60)	P10809
EBNA-1 NUCLEAR PROTEIN	P03211
IR-3, Internal Repeat Region (in EBNA-1 e.g. Proteins)	
Chitinase-3 like protein 1 precursor (Cartilage glycoprotein-39) (GP-39) (39 kDa synovial protein) (YKL-40)	P36222
Collagen alpha 1(II) chain precursor [Contains: Chondrocalcin]	P02458
CH65, Chondrocyte Antigene 65	

Collagen-binding protein 2 precursor (Colligin 2) (Rheumatoid arthritis related antigen RA-A47)	P50454
47 kDa heat shock protein precursor (Collagen-binding protein 1) (Colligin 1)	P29043
Chitinase 3-like protein 2 precursor (YKL-39) (Chondrocyte protein 39)	Q15782
Chitinase 3-like protein 2 precursor (YKL-39) (Chondrocyte protein 39)	Q15783
Chitinase 3-like protein 2 precursor (YKL-39) (Chondrocyte protein 39)	Q15749
Fructose-bisphosphate aldolase A (Muscle-type aldolase) (Lung cancer antigen NY-LU-1)	P04075
Proteoglycan link protein precursor (Cartilage link protein) (LP)	P10915
Matrix metalloproteinase-19 precursor (MMP-19) (Matrix metalloproteinase RASI)	Q99542
MMP-19 (matrix metalloproteinase)	CAA63299
Aggrecan core protein precursor (Cartilage-specific proteoglycan core protein) (CSPCP) (Chondroitin sulfate proteoglycan core protein 1)	P16112
Ezrin (p81) (Cytovillin) (Villin 2)	P15311
Radixin	P35241
Moesin (Membrane-organizing extension spike protein)	P26038

The invention will now be described by means of examples, however without being limited to them.

Examples

Example 1: Employment in clinical diagnostics

A patient, having articular symptoms for 4 month, suffers from an asymmetric swelling and painfulness in 2 proximal joints and 1 middle joint of the finger and in the right wrist joint. The stiffness in the morning persists for about 30 minutes. The radiological picture shows a beginning erosive alteration in one proximal joint of the toe. The C-reactive Protein is within the normal range, the sedimentation rate is slightly increased, rheumatoid factor and HLA-DR4 are negative. There is no familiar history concerning an inflammatory rheumatoid disease.

During an ambulant appointment, a synovial biopsy from the right wrist joint was isolated by minimally invasive arthroscopy. Of four samples having a weight of a about 10 mg each, a

little sample is fixed in formalin for the following histological evaluation. The remaining samples are introduced into RNA lysis-buffer, crushed up and the RNA is extracted according to standard protocols. After the (reverse) transcription into cDNA, an in vitro transcription into a biotin-labelled cRNA constituting a transcription of the cDNA, is performed. The cRNA is fragmented and then employed for the hybridisation to the DNA-array.

The array is produced by a commercial company for the generation of DNA-arrays, like e.g. Affymetrix. There, suitable oligonucleotides are deduced from the sequences of table 1 and from the gene sequences coding for the proteins of table 2, whereat these oligonucleotides allow for a specific hybridisation to the respective cRNA-sequences. These sequences are either synthesized as oligonucleotides and then printed onto an array-carrier, or they are directly synthesized on the carrier, e.g. by a photolithographic method.

The hybridisation is performed according to the instructions of the manufacturer's protocol. The DNA-array is read by means of a scanner. The translation of the optical information into expression signals is accomplished by using standard software, like e.g. "Micro-Array Suite" from Affymetrix. One now has obtained the signals of the RNA expression rates of the genes or proteins mentioned in the tables 1 and 2. Starting from this newly defined selection of genes for the diagnostic evaluation and therapy development for joint diseases, clinically and histologically characterized tissue samples were classified and related to each other in a hierarchical manner after cluster analysis during preliminary tests. Due to the comparative association with the clinical findings, this classification was accomplished in particular in dependence on the type of disease (arthrosis, reactive arthritis, rheumatoid arthritis, subgroups of rheumatoid arthritis), the activity of the disease und thus the prognosis and the possibility of affecting the pathologically altered gene expression by means of an applied drug. The signal data of the above mentioned patient are then compared to this database. Thereby, an assignment to one of these groups becomes possible, and one can obtain information about the corresponding clinical associations. Thus, one obtains evidence about the diagnosis, the activity, the prognosis and the therapeutic options in the individual patient.

Example 2: Employment for the evaluation of therapies

A patient, who has been suffering from a chronic joint inflammation for 5 years, diagnosed as a rheumatoid arthritis, shows progressive specific radiological changes in several finger joints,

accompanied by pain and swelling in several finger joints, the left elbow joint and the right ankle joint despite a current basal therapy under application of 15 mg of Methotrexate per week. During an ambulant appointment, a synovial biopsy from the left elbow joint was isolated by minimally invasive arthroscopy. Several samples of about 30 mg total weight were introduced into lysis-buffer, crushed up and the RNA was extracted. The preparation of the sample was accomplished in a similar manner as in example 1. The same DNA chip like in example 1 is used for analysis. After hybridisation, the transfer of the results of hybridisation into a picture data file and translation of the results into signal information for each of the tested genes, an assignment to defined expression pattern is accomplished. These patterns were determined in preliminary tests, thereby using the defined selection of genes from table 1 and 2 being newly defined in this specification. Thereby, the alteration of the expression profile of a sample was analysed in dependence on the respective joint disease, which is affected by defined drugs applied in defined concentrations. The profiles were hierarchically classified, thereby considering the association with the employed drugs and the applied dose. When the patient sample is compared to these defined expression patterns, the assignment to a specific pattern and the therapeutic efficiency information associated therewith make it possible to estimate, if the applied drug Methotrexate could be effective at a higher dose, or if it is reasonable to change to a drug, the activity profile of which fits best for affecting the pathological changes in the individual case.

Example 3: Autoreactivity profiles in the RA

The RA is different from other rheumatic and other inflammatory diseases in respect of the generation of auto-antibodies. Thereby, a distinction between RA and non-RA is not provided by one antibody-reactivity, but by different profiles of several autoreactivities. It is thus possible to obtain save diagnostics, to control therapeutic progress and to perform preventive examinations based on the determination of the RA-specific autoreactivity profiles.

Antibodies are directed against antigens, or, more precisely, against epitopes, which are bound by the paratopes during a specific antibody-antigen-reaction. An epitope is defined as the region of an antigen, which specifically interacts with an antibody (i.e. with its paratope). In general, an epitope is understood as a peptide sequence of a protein, whereat this peptide sequences comprises about 16 to 20 amino acids. This sequence can be consecutive (continuous epitope) or interrupted (discontinuous epitope). Typically however, there are only

a few amino acids, in rare cases just one amino acid, necessary and sufficient for the specific interaction between antibody and antigen. Meanwhile, it is known, that even nucleic acids can act as antigens. Particular importance is more and more attributed to posttranslational modifications like e.g. phosphorylation, acylation, glycosylation, methylation, deimination, etc. Since these modifications often have a regulatory function, they seem to be particularly interesting as target structures of antigens, especially under pathological conditions. Since it has already been shown for some RA-associated auto-antigens, that specific post-translational modifications produce epitopes for auto-antibodies, it has to be paid particular attention thereupon, that these structures are realized in the test system.

The proteins listed in table 2 have been described as RA-associated auto-antigens. The relevance of most of these single components however, is low or not obvious for the diagnostics of RA. The same applies to the genes being overexpressed on the mRNA level, which are listed in table 1. These components by themselves are not suitable to significantly improve the diagnostics of RA. This is shown by the fact, that practically the majority of the proteins listed in tables 1 and 2 are not applied for as patents for this respective purpose. Only a few proteins are such characteristic, that a relevance for RA has been assumed. This is e.g. valid for the protein BiP (Heavy Chain Binding Protein), which is the target of an immune reaction in RA. Here, e.g. a post-translational modification in the form of a glycosylation has to be taken into account, since this modification is a component of epitopes, which are both necessary for the recognition of auto-antibodies in RA, and for the distinction between RA- and non-RA-auto-antibodies. Moreover, the amino acid being post-translationally transformed from arginine to citrullin was described as an essential epitope for RA-associated auto-antibodies (6). A similarly high significance for the diagnostics of RA is valid for the Sa-antigen (5), the RA33-antigen and for Calpastatin.

Nevertheless, these components by themselves were not appropriate to allow for an unambiguous diagnosis of RA or even for the monitoring of a therapy. The depicted, novel approach according to the invention refers to the immunome of RA. The immunome of RA comprises the entirety of autoreactive antibodies, which are present in RA, and also the entirety of the auto-antigens or auto-epitopes recognized by these antibodies. Unexpectedly, it was able to be found, that it is possible for the first time to diagnose a disease unambiguously as an RA by analysing the combination of RA-associated auto-antibodies. It was able to be shown for the first time, that there exist different patterns of auto-antibodies, which

exclusively occur in RA. These patterns also include such auto-antigens or autoreactivities, which by themselves appear to be unimportant for the RA. These is even more surprising, since respective first approaches of other groups did not lead to this finding, although it is emphasized, that the most important auto-antigens from eight different human autoimmune diseases were employed (11). The same applies for an approach, in which auto-antigens were used, which are relevant for another rheumatic disease, the systemic Lupus erythematodes (SLE). Apparently, the essential difference between the approaches already being published and the approach described herein, is on the one hand based on the type of analysis (multivariate), on the other hand on the composition of the auto-antigens. Only a sufficiently high number of RA-relevant auto-reactivities allows for an unambiguous diagnosis. Thus, the entirety of the RA-associated auto-antibodies and auto-antigens constitutes information, which - together with other techniques (protein array technology (27), data processing) - can be, among other applications, employed as a means for the diagnostics and classification of RA. Even an expert in this field would not have been able to conclude such a use degree by means of analogy deduction. The immunome of RA and also mere parts of the RA-immunome can be employed for unambiguously distinguishing RA from other diseases or from the healthy state. A commercial utilization of the unexpected invention moreover only becomes possible by the currently available or still developing possibilities of the high-throughput technologies. This refers in particular to the multiple-parameter-analysis of autoreactivities, since it is necessary in this place, to perform a multiplicity of parallel analyses under the employment of miniscule sample sizes derived from the patient.

Proteins or partial protein sequences of the components given in table 2, or proteins and partial protein sequences encoded by genes given in table 1, including the post-translational modifications being potentially necessary for the distinction between RA and non-RA, are synthesized and provided for the generation of autoreactivity profiles. The synthesis can be accomplished by an arbitrary, known approach based on molecular biology or by an arbitrary approach of protein chemistry. Furthermore, partially artificial (in vitro translation) or artificial synthesis according to the state of the art are suitable to produce said proteins or partial protein sequences.

Protein Array / Peptide Array (28)

Proteins or partial protein sequences according to table 2 or 1 are used in their entirety or only as a respective selection suitable for the immunomic distinction of clinical pictures, in order to create a test option, which is suitable to determine the autoreactivities of an individual. This particularly refers to the selection of Citrullin, BiP, p205, IgG, Calpastatin, RA33, Sa-antigen and Calreticulin. For this aim, the proteins are separately applied to a carrier matrix at positions allowing for a spatial resolution. The position and identity of each immobilized protein, peptide, modified protein or modified peptide are known. The micro-format allows for a parallel detection of thousands of different antigens and/or auto-antigens (proteins/peptides) in the sub-microliter range of human sera. Preferred options are the preparation of a Protein Array, of a high-density filter, of a high-density glass carrier or of another matrix produced by the high-density method, whereat this matrix in a coated or non-coated form is coupled to proteins or partial protein sequences. For instance, proteins or partial protein sequences can be printed onto derivatized or coated/activated glass carriers, or the application is accomplished by means of the ink jet-method, in a capillary manner, or by direct synthesis on the array under the employment of photolithographic masks or digital micro-reflectors. Instead of glass carriers one can also use membranes and filters, polystyrene matrices, Nanowell-plates and micro-particles (29).

The Protein Array is incubated together with a suitable dilution of patient sera or as well of patients' joint effusions. During this incubation, possibly present antibodies having specificity for one or several protein components can bind to these protein-antigens. This is followed by a washing step in order to remove remaining free antibody and serum components. Then one incubates the sample with a second antibody, which is suitable both to indicate a successful antigen-antibody-reaction by binding the first antibody and to introduce a suitable label, which allows for visualization and quantification, suitably a covalently coupled fluorescence dye or a covalently coupled enzyme being capable to produce a dye from a precursor substance. This is followed by a further washing step in order to remove the remaining free second antibody.

Suspension Array (30)

The Suspension Array uses plastic particles as a matrix, whereat the plastic particles are coated with the mentioned proteins. This is such accomplished, that the optical characteristics of particles coupled to a specific protein are different to the optical characteristics of particles

coupled to another protein. The immunomic analysis is performed in an analogous manner by the incubation with patient sera or other bodily fluids. By means of the antibody-reaction with a suitable second antibody, a further optical (fluorescent) signal is produced either directly or again indirectly. The analysis is then performed in a multicolor-fluorescence activated cell (FAC-) scan.

Time-resolved Protein Arrays (31)

A polystyrene surface is coupled to different proteins or partial protein sequences taken from table 1 and 2. The antibodies to be analysed from the patient sera are biotinylated by using an active biotin-ester. Alternatively, one may also use biotinylated secondary antibodies being specific for human antibodies in order to avoid inter-patient-deviations in consequence of a different efficiency of biotinylation. The patient antibodies are then incubated with the protein-coupled polystyrene surface. After a subsequent washing step, the detection is accomplished by means of Streptavidin, which is coupled to a fluorescent Europium complex. The evaluation is then accomplished after a washing and drying step by means of laser-excited, time-resolved solid phase fluorescence analysis.

Data patterns and multifactor analysis

Parameters (e.g. the autoreactivities obtained for the proteins/auto-antigens listed in tables 1 and 2; e.g. the autoreactivities RF/ Citrullin/ BiP/ Calpastatin/ Calreticulin/ RA33) are determined as complete as possible. Data patterns of individual patients having more than 2 of 6 missing values were *a priori* excluded from the analysis.

The interpretation of the immunodetection system yields a negative or positive result for each patient and each auto-reactivity. An alternative option are continuous values (Protein Array, ELISA), which are divided into positive or negative either artificially (mathematically) or by a control group-related Cut Off (analysis in comparison to a suitable control group, e.g. age- and sex-matched healthy controls or control-patients suffering from another disease). Each data pattern is analysed and classified by means of the CLASSIF1 program system (32).

In a first step, the triple-matrix characters of each clinical diagnosis category are entered into the first reference classification mask. Each patient is then classified according to the highest degree of position identity between the patient mask and a clinical reference mask.

In a second step, those data columns are eliminated, which display the triple-matrix character "0" for all reference masks, since they do not allow for a distinction between the disease entities.

In a third step, the CLASSIF1-algorithm transiently eliminates either individual parameters or combinations of two parameters in all permutations from the classification process. The total data set is then reclassified. Parameters, which affect the classification result by their transient elimination, are informative, since obviously no essential information is lost. The information content of each parameter is intermittently provided by the algorithm, reintroduced after the operation and the next parameter or the next pair of parameters is transiently extracted and analysed in an analogous manner. The intermittent removal and reintroduction is performed, until the information content of all parameters, either alone or in combination, is revealed. Parameters, which prove to be uninformative either alone or in combination with a further parameter, are eliminated. The remaining sequence of informative parameters constitutes the reference classification mask for the respective clinical prediction category.

In a fourth step, the classification is optimized by classifying the percentile Cut Off values 10/90%, 15/85%, 20/80%, 25/75% and 30/70% with the subsequent selection of the pair showing the best discriminating properties. The best classification results are typically reached in the range between the 10/90% and 25/75% percentile pairs. Negative and positive predictive values in a Confusion Matrix provide information about how good the reference sample and the samples to be tested are discriminated by the employed pattern(s).
Additionally, the data patterns of each patient are subjected to a multifactor analysis. The multifactors for five parameter patterns were obtained by multiplication or division of the different parameters in all possible combinations, followed by the standardization of the five data columns towards the mean values of the RA-reference group. Subsequently, the mean values for each parameter of the other patient groups (e.g. OA, reA, PsoA, other) were determined. Multifactors for all parameter permutations were either determined by multiplication, when the parameter's mean value of the respective patient group was increased in comparison to the reference value (RA), or by division, when the value was decreased.

The multifactor database comprises the measured parameters (RF/ Citrullin/ BiP/ Calpastatin/ Calreticulin/ RA33). 26 multifactors have been classified via the CLASSIF1-algorithm. Thereby, all figures of each database column were transformed either into "-" (less than the lower percentile of the value distribution of the reference patients [RA]), "0" (between the lower and upper percentile) or "+" (larger than the upper percentile) triple-matrix characters. Following the transformation of the database columns, a confusion matrix is established between clinical diagnosis and computer classification.

The diagonal values of this confusion matrix represent the specificity of the reference samples and the sensitivity of the samples to be tested. These are further optimized during the subsequent iterative learning process. An optimal classification is achieved, when all samples have been correctly classified, that is when all diagonal values of the confusion matrix reach 100% and the values of the non-diagonal fields are 0%. The learning process serves to eliminate non-informative parameters and thus to accumulate the discriminating parameters.

Description of the Figure

Fig. 1: Autoreactivity pattern with RA33, RF, Citrullin, BiP and Calpastatin

Depicted are all 32 possible combinations of the autoreactivities against IgG (RF), Citrullin, BiP, Calpastatin, RA33 and Calpastatin for the disease entities RA (rheumatoid arthritis), reA (reactive arthritis), OA (osteoarthrosis), PsoA (psoriasis-associated arthritis) and other.

List of abbreviations

ACR	American College of Rheumatology
BiP	Binding Protein, Heavy Chain Binding Protein
BSA	Bovine Serum Albumin
Calp	Calpastatin
Calr	Calreticulin
cDNA	complementary DNA, copy DNA
CH	Chondrocyte Antigen
Cit	citrullinated peptide

CrP	C-reactive Protein
DNA	desoxyribonucleic acid
DPNII	from Diplococcus pneumoniae
dNTP	desoxynucleotide-triphosphates (equimolar mixture of dATP, dCTP, dGTP, dTTP)
dNTP	desoxynucleotide-triphosphate
EBNA-1	Epstein-Barr virus nuclear antigen-1
EBV	Epstein-Barr virus
ER	endoplasmatic reticulum
FACS	Fluorescence Activated Cell Sorting
GAPDH	Glycerol-aldehyde-phosphate dehydrogenase
HC	Human Cartilage
HC gp39	Human Cartilage glycoprotein 39
HLA-system	histocompatibility antigen (HLA - human leucocyte antigen)
HLA-DR4	HLA feature, that exhibits an increased association with a rheumatoid arthritis
hnRNP	heterogeneous ribonucleoprotein (RA33)
Hsp	Heat shock protein
Ig	immunoglobulin
IgG	immunoglobulin G
IL-	interleukin
IR-3	internal repeat region 3
MCTD	Mixed Connective Tissue Disease (mixed collagenosis)
MHC-	Major Histocompatibility Complex
MMP	matrix metalloproteinase
mRNA	messenger ribonucleic acid
NAD	nicotineamide-adenine-dinucleotide
NCBI	National Centre for Biotechnology Information
ND	normal donor
OA	osteoarthrosis
O-GlcNAc	O-N-acetylglucosamine
PCR	Polymerase Chain Reaction
PHA	phytohemagglutinin
PM/DM	polymyositis/ dermatomyositis

PsoA	psoriasis-associated arthritis
RA	rheumatoid arthritis
RA-A47	arthritis-related antigen
RA33	hnRNP A2
RDA	Representational Difference Analysis
reA	reactive arthritis
RF	rheumatoid factors
RNA	ribonucleic acid
RPMI	commercially available, conventional cell culture medium, dilution Medium RPMI 1640; (Moore, G.E. et al., J. Am. Assoc. 199, 519-524, 1967)
RsaI	DNA restriction enzyme RsaI from Rhodopseudomonas sphaeroides
RT	Reverse Transcriptase (RT)
Sa-antigen	50k-protein from human spleen and placenta
SLE	systemic Lupus erythematodes
SSH	Suppression Subtractive Hybridisation
TGF	Transforming Growth Factor
UNIGENE	UniGene is an experimental system for the automatic partition of the GeneBank-sequences into a non-redundant set of gene-orientated Clusters
YKL-39	Human Cartilage Related Protein

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